THE HAWAIIAN PLANTERS' RECORD

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A quarterly paper devoted to the sugar interests of Hawaii and issued by the Experiment Station for circulation among the Plantations of the Hawaiian Sugar Planters' Association.

In This Issue:

Molasses and Biological Soil Activity:

A study of the action of molasses in the soil shows it to be to a great extent biological. The sugars contained in the molasses disappear rapidly due to the growth of yeasts. In this process a large amount of living micro-organic tissue is built up and remains in the soil as such. Nitrate or inorganic nitrogen is changed to the organic form as protein in the yeast cells. Approximately 25 pounds of nitrogen is used per ton of molasses applied.

Forestry in Formosa:

A few paragraphs and photographs pertaining to the forestry problem in Formosa are presented. The article describes the forests, the amount of lumber obtained as well as a few species of trees that occur in the forests. The photographs give some idea of the trees and of the reforestation work that is being carried out by the Government.

Cane Diseases in Mauritius:

The salient points regarding cane diseases that occur in Mauritius are briefly summarized from the Annual Report of the Department of Agriculture of the Colony of Mauritius for the year 1928.

Leaf scald disease was recorded for the first time in Mauritius. Other interesting points are brought out on the following diseases: leaf scald, gumming disease, red rot, pineapple disease, smut, streak disease, root disease complex, pokkah boeng, eye spot, stem deterioration and the cane-killing weed.

Methods in Plant Physiology:

An account is given of methods in plant physiology, utilizing sugar cane in water culture.

Forestry on Oahu:

A review is presented of ten years participation in forestry work on Oahu.

Pre-Harvest Sampling of Cane:

By courtesy of a committee of the Association of Hawaiian Sugar Technologists this issue presents a study of the pre-harvest sampling of cane by refractometer at Ewa Plantation Company, which indicates that the progress of cane ripening can be followed in a general way through pre-harvest sampling for the determination of solids only.

Modification of the Kjeldahl Method:

A modification of the standard method for the determination of Kjeldahl nitrogen saves much time without any sacrifice of accuracy. This modification consists of the substitution of boric acid for standard sulfuric acid as the absorbent of the ammonia. In this article the results of applying the new methods to the determination of nitrogen in fertilizers at this Station is discussed.

Decomposition of Carbohydrates in Water-logged Soils:

A review is presented of a recent paper dealing with the fermentation of sugar in water-logged or water-covered soils, the effect on fermentation of additional elements, the transitory effect in the soil, the various steps involved in the decomposition and the final products of such anaerobic decomposition of carbohydrates.

Determination of Phosphoric Acid Colorimetrically:

A large number of determinations of phosphoric acid are made in the laboratory of the chemistry department. The standard method of analysis is slow and somewhat tedious. A colorimetric method is described which is rapid, accurate, and in many respects superior to the older method generally in vogue.

Studies on Plant and Water Relations in Hawaii:

That Hawaiian soils possess a more or less precise wilting coefficient, and that local soils are similar to those found elsewhere in that the residual moisture content at the time of wilt depends only upon the soil and not upon the sort of plant under observation, is suggested by the report of a series of experiments included in this issue.

Waipio soil in carefully sealed containers was planted to sunflowers and to buff beans. The residual soil moisture at the time of wilting was surprisingly similar. Although two species are too few to permit general conclusions they do provide some evidence that local soils are similar to mainland soils in the possession of a definite wilting coefficient. The percentage of soil-moisture at the time of wilt was remarkably high. Plants in the soil under observation showed real wilt at a moisture content greater than that contained in many mainland soils immediately after irrigation.

Difficulties were experienced when sugar cane plants were used as an indicator of wilt. Cane apparently does not wilt in the sense used in the literature of the subject. The leaves consistently regain turgidity after a given exposure to a saturated atmosphere, regardless of the soil-moisture content. However, physiological disturbances may be noted at the moisture content identified as the wilting coefficient by other plants. The most evident disturbance is a reduction in the rate of transpiration after the wilting coefficient has been reached. Studies since the completion of the original paper appearing in this issue indicate that elongation or growth is impossible when the soil moisture is below this critical constant.

This work is being continued and elaborated at Waipio.

Germination of Sugar Cane Pollen:

The germination of sugar cane pollen is dependent upon a nice balance of retroactive physical and chemical factors. Experiments are reported whereby an attempt is made to standardize a method of germinating sugar cane pollen *in vitro* in hanging drops of culture media by controlling the concentrations of sugar and agar and the pH values of the media, the relative humidity and the temperature of the air surrounding the hanging drops, and by addition of enzymes to the culture media.

Quantitative data are offered to show that, when a series of culture media having a gradient of viscosities due to increased concentrations of sugar and agar was used, one particular medium consistently gave the maximum percentage of germination. An optimum relative humidity and temperature were found. While there was a correlation between temperature and percentage of germination there also existed a correlation between percentage of germination and the difference between the temperature of the air when the pollen was shed and the temperature at which germination occurred. The addition of Taka-diastase to the culture media definitely increased the percentage of germination.

Methods in Plant Physiology

By H. L. Lyon and Hugh Brodie

The investigation of certain problems in the physiology of the sugar cane plant through the application of precise laboratory methods was a new line of work allotted to the department of botany and forestry at the beginning of the year 1929. In the few months that have elapsed since that time, all of our efforts in this particular field of research have been directed towards the development of a suitable technique, so we have as yet no contributions to the knowledge of the physiology of the sugar cane plant to offer. It seems, however, that one of our experiments being carried on in the open at the Experiment Station has attracted considerable attention and the present paper is written to satisfy numerous demands for a description of the technique employed.

We are striving to develop methods for the study of water consumption by the cane plant and the experiment in question represents one of the several methods which we are testing out. In this particular experiment, a plant was grown in water culture with its root system confined within a sealed container into which new solution was admitted from an outside reservoir to replace that withdrawn by the plant. By a system of automatic weighing and recording, the speed of water consumption was indicated on a chart.

The shape and construction of the container employed and the method of installing a cane plant therein is indicated in the diagram reproduced herewith as Fig. 1. This container is made in two parts: a cylindrical tank 21 inches deep by 12 inches in diameter with a flange on its rim and a conical cover, also flanged on its rim to match the flange on the tank. A tight seal between the cover and tank is secured by interposing a waxed gasket between their flanges and bringing these tightly together with bolts spaced about 1 inch apart. A tight seal about the stalk of cane in the neck of the container is secured by inserting a large rubber stopper, "R," accurately bored to fit the stalk. This is forced tightly into place and covered, first with a layer of wax and then with a poured plug of a cement made of litharge and glycerine, which is eventually coated over on its exposed surface with shellac. A socket, "S," centrally attached to the bottom of the tank serves to hold the base of the cane stalk in position in the container. Three tubes, "I," "A" and "O," through the cover serve as an inlet for solution, an outlet for air and solution and an avenue through which thermometers and other instruments may be lowered into the tank. These tanks are made of 24-gauge galvanized iron and the tubes are of brass. All exposed surfaces on the interior of the tank are coated with paraffine when it is about to be used.

A suitable cane plant for experiment is secured by selecting an average stalk from a stool in the field and causing it to throw out roots at the desired level by encasing this portion of the stalk in a jacket filled with sand, moss or sawdust, which is kept moist by adding water at frequent intervals. A portion of the inner tube of an automobile tire slipped on over the top of the shoot and tied up at its

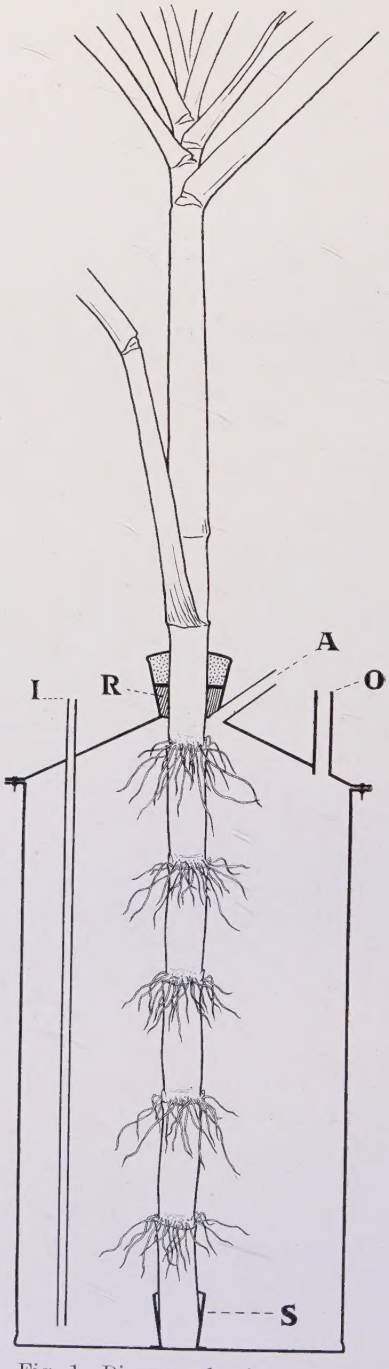


Fig. 1. Diagram showing container in vertical section. For description see text.

Fig. 2. Cane stalk encased in a piece of inner tube filled with moist sawdust to induce growth of roots. Photo by E. L. Caum.

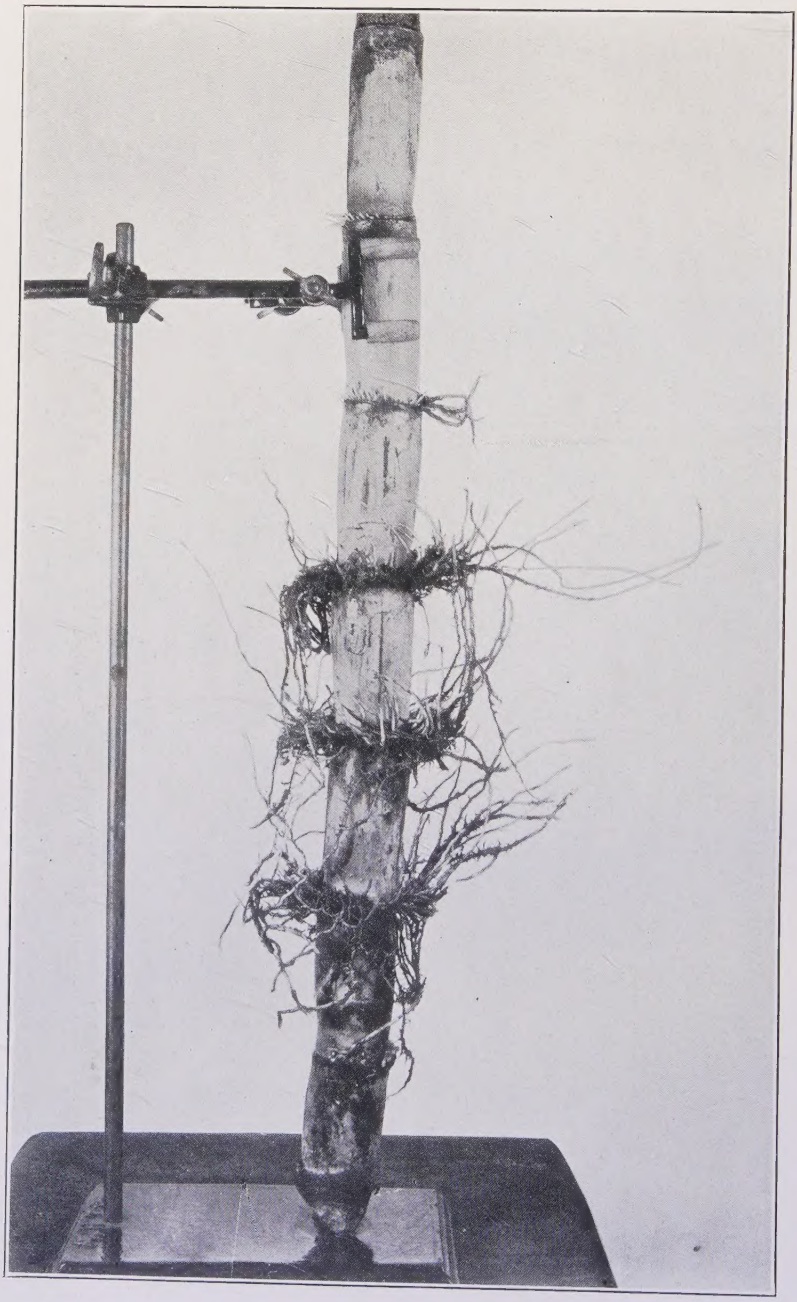


Fig. 3. Root system of cane stalk at time of transfer from field to sealed container. Photo by E. L. Caum.

Fig. 4. A cane plant which has been grown for nine months with its root system enclosed in a sealed tank, this tank standing in a larger container through which tap water is kept flowing to regulate the temperature. Photo by Twigg-Smith.

lower end in the manner indicated in Fig. 2 makes a very serviceable jacket. A cane stalk being treated in this manner is staked up to keep it from falling over under the added weight. When a cane stalk so jacketed has developed a goodly number of roots, it is cut off in an appropriate length and transferred to culture solution in a sealed container in the manner already indicated, the jacket and absorbent material being first removed. (See Fig. 3.) This method of transferring a fully developed cane shoot from a stool in the field to a water or soil culture is by no means new. Its employment in sugar cane breeding work has been well described and illustrated by Venkatraman and Thomas.*

While a cane plant is growing in a sealed tank as described above, the solution within the tank is held at a reasonably constant temperature by placing the tank within a larger container through which tap water is kept flowing at all times. By this means, variation in temperature may easily be kept within 2 degrees C. in any 24-hour period.

We have grown a cane plant in a tank of this sort for a period of 12 months and it made a growth quite equal to, if not better than, that displayed by the other shoots of the stool from which it was taken. This particular plant is illustrated in Fig. 4, the picture being taken when it was about 9 months old. The original shoot elongated quite as rapidly as did its sister shoots, which remained in the field, but in addition to this elongation, it threw out lalas at an early age which eventually developed into sticks of considerable size. At the proper season, this plant flowered in the normal manner and later elaborated a tassel in the top of one of its lalas. After removal from the stool in the field, this cane shoot received tap water only throughout the entire year in which it was growing in the tank. No salts whatsoever were added at any time, the plant extracting all of its nutrients from tap water which, at the Experiment Station, is from artesian wells.

The performance of this plant under the conditions to which it was subjected excited much interest and comment among observers and opinions were freely offered regarding the composition of the cane and the quality of its juices. When it had completed a full year's growth on tap water in tank culture, it was taken out and numerous analyses secured, the more interesting results being recorded in the accompanying tables. When the sealed container was opened, it was found to be packed full of roots which were interlaced into a compact mass that retained the shape of the container after it was withdrawn therefrom. The extent and condition of the root system are well illustrated in Figs. 5 and 6.

JUICES OF CANE GROWN IN TAP WATER

	Brix	Polarization	Purity	Quality Ratio
Main stalk	19.66	17.97	90.86	7.3
Lalas	19.17	17.46	91.08	7.49

Analyses by H. F. Bomonti

* *The Agricultural Journal of India* 21:203-209, 1926.

Fig. 5. Root system of cane plant shown in Fig. 4 when removed from sealed container at end of twelve months. Photo by E. L. Caum.

Fig. 6. Same subject as Fig. 5 but with one-half of root system cut away. Photo by E. L. Caum.

COMPOSITION OF CANE GROWN IN TAP WATER COMPARED WITH THAT OF CANES OF THE SAME VARIETY GROWN IN THE FIELD

Date of Sampling	Origin of Sample	Age Months	Part of Plant	Percentages Expressed on Dry Plant Substance				Percentages Calculated to Green Plant Basis				
				Organic Matter Sugar, etc.	Ash Per Cent	Silica SiO_2 Per Cent	Nitro. N Per Cent	Moist. Per Cent	Organic Matter Sugar, etc.	Ash Per Cent	Silica SiO_2 Per Cent	Nitro. N Per Cent
Mar. 12, 1930	H 109 from Transpiration Experiment at Experiment Station	12	Lala Leaves	91.0	9.01	4.84	.95	72.49	25.03	2.478	1.330	.261
			Lala Stalk	97.8	2.21	1.08	.69	71.16	28.20	.636	.311	.199
			Main Stalk	97.8	2.20	1.09	.43	71.12	28.25	.634	.315	.125
Jan. 13, 1930	H 109 from Oahu Sugar Co., Ltd., Field 49	22 (Est.)	Leaves and Tops	92.5	7.47	1.68	.88	80.03	18.48	1.492	.335	.176
			Stalks	99.1	.92	.17	.15	74.88	24.89	.231	.043	.038
Feb. 10, 1930	H 109 from Waimanalo Sugar Co. Field 175	12	Leaves and Tops	92.7	7.35	2.02	1.08	77.25	21.08	1.672	.460	.245
			Stalks	97.4	2.56	.19	.31	77.71	21.72	.571	.042	.070

Analyses by J. H. Duffy

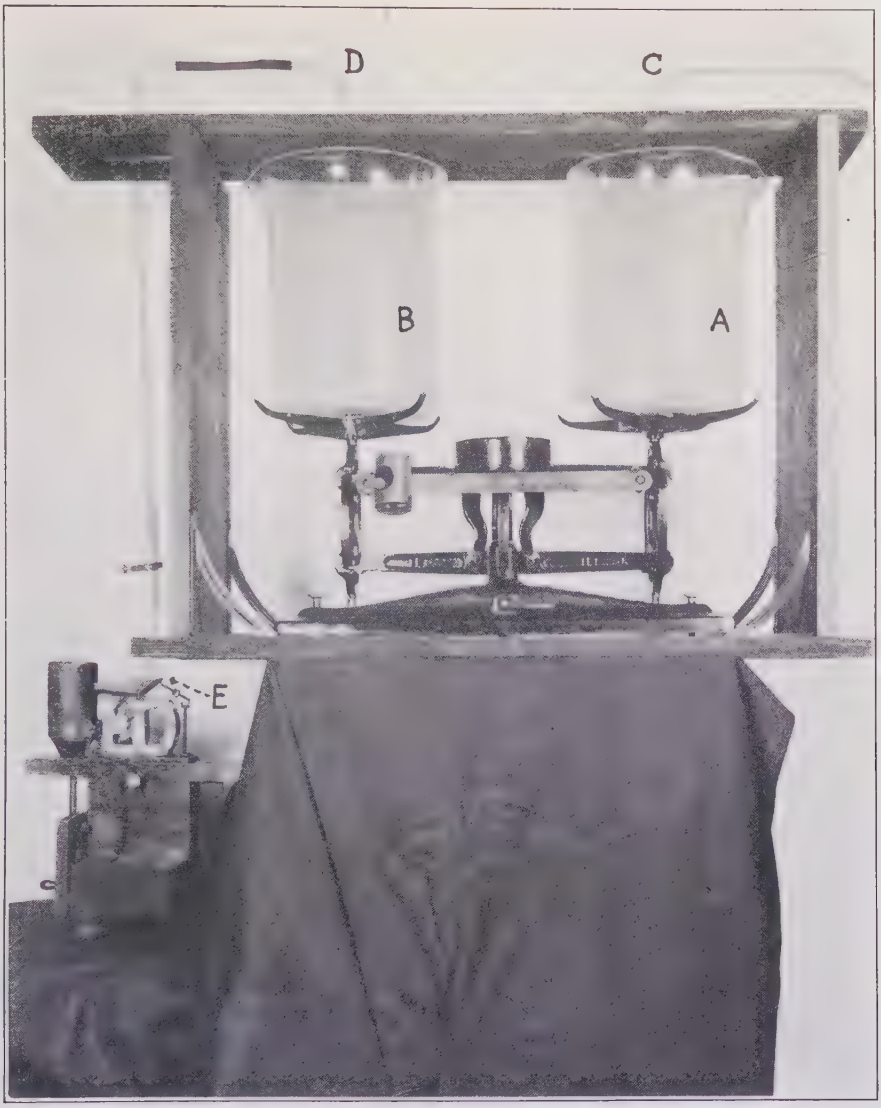


Fig. 7. Apparatus for the automatic weighing of water withdrawn from container by cane plant. For description see text. Photo by E. L. Caum.

As previously indicated, the sugar cane plant described and illustrated above was employed as a subject in the evolution of a technique for determining the rate of water consumption by a cane plant and we will now describe this technique in its present stage of development. During periods of observation, water extracted from the container by the plant is replaced from a reservoir through a rubber tube connecting with the brass inlet tube "I," shown in Fig. 1. The supply reservoir is held at a slight elevation above the culture tank so that a flow from the reservoir into the tank can be maintained by gravity.

Now, in preparing to take a reading on the rate of water consumption, tubes "A" and "O" are opened and water is allowed to flow into the tank through

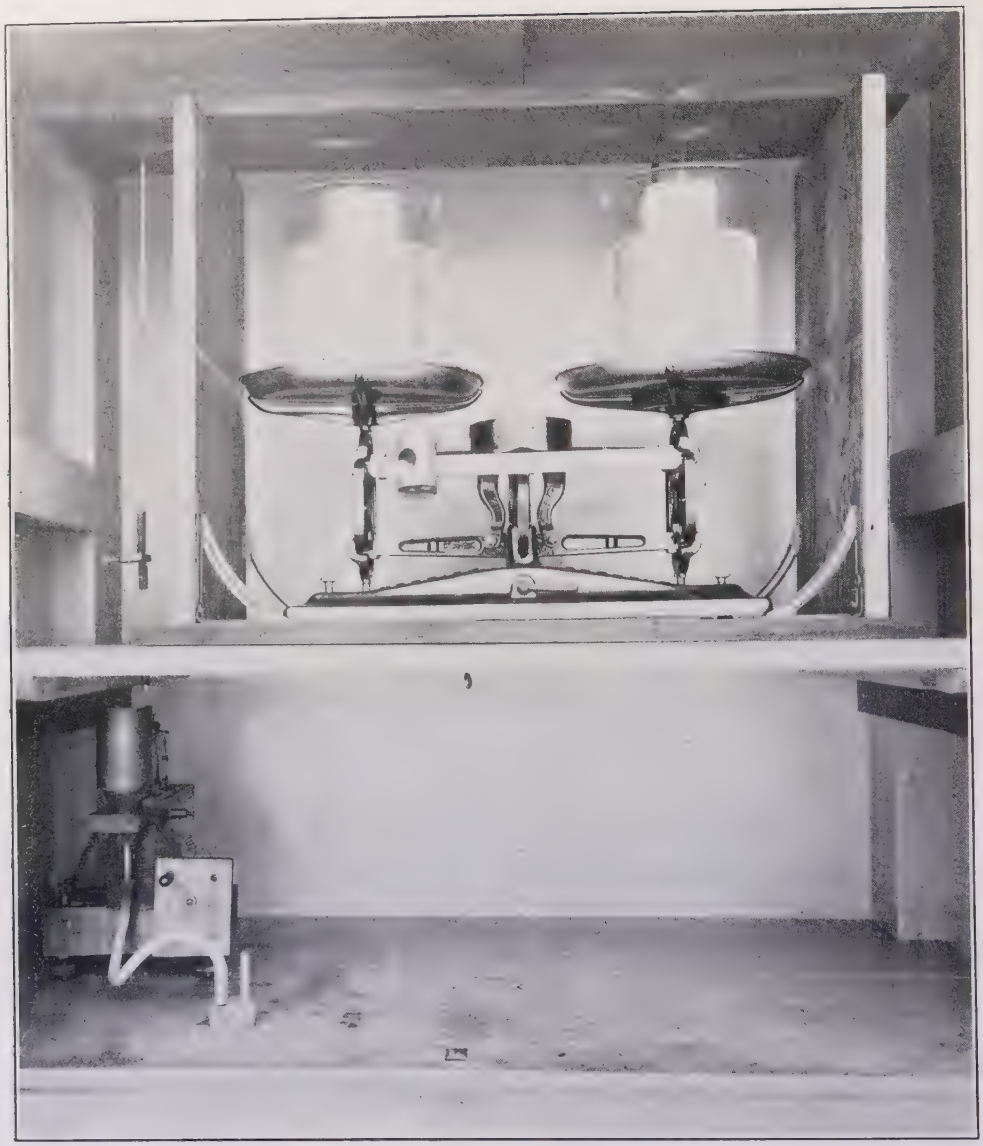


Fig. 8. Weighing apparatus installed in cabinet in the field. At the time the picture was taken, one siphon was closed and the balance was blocked to prevent movement.

tube "I." Such air as may be in the tank will be forced out through tube "A" and when water is flowing from "A" and "O," both of these tubes are securely closed with stoppers. From that time on, water passing in through "I" will represent that consumed by the plant, and the speed of water consumption is the speed of outflow from the supply reservoir. Our apparatus for measuring the flow of water from the reservoir into the tank is demonstrated in the set-up shown in Fig. 7. Two beakers—"A" and "B"—of equal capacity and of approximately equal weight are placed upon a Torsion balance within a rack, as illustrated. Tubes "C" and "D," of equal size and supported by the rack, extend down nearly to the bottoms

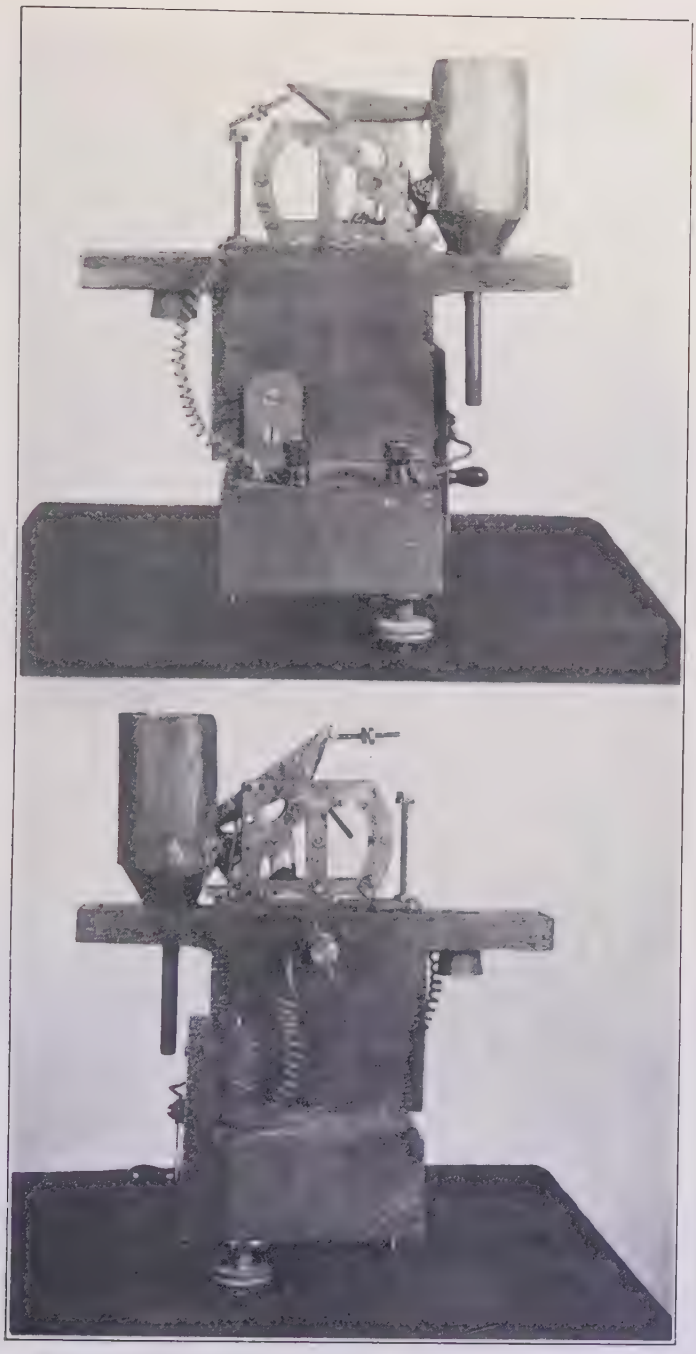


Fig. 9. Trip-bucket scale used in experiments with cane plant shown in Fig. 4. Photo by E. L. Caum.

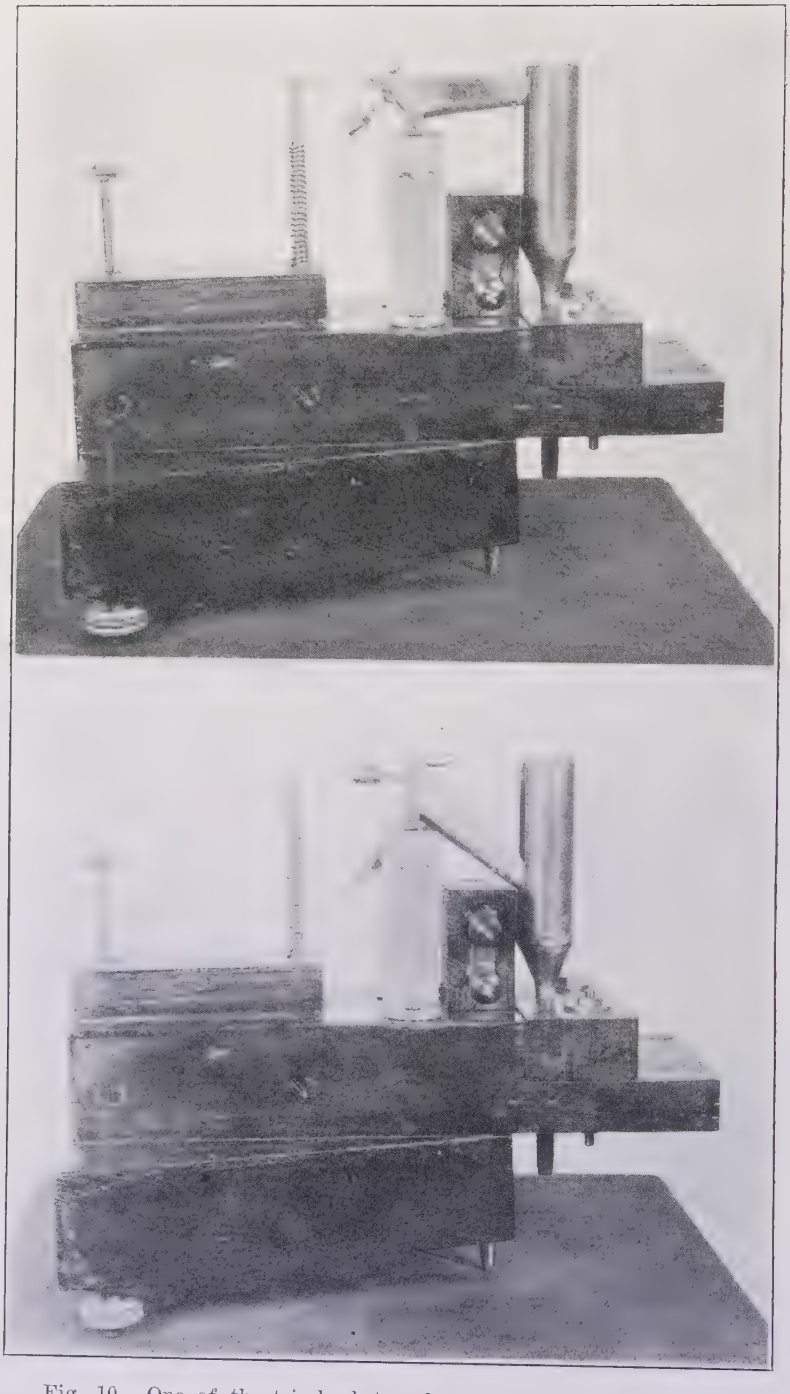


Fig. 10. One of the trip-bucket scales now in use. Photo by E. L. Caum.

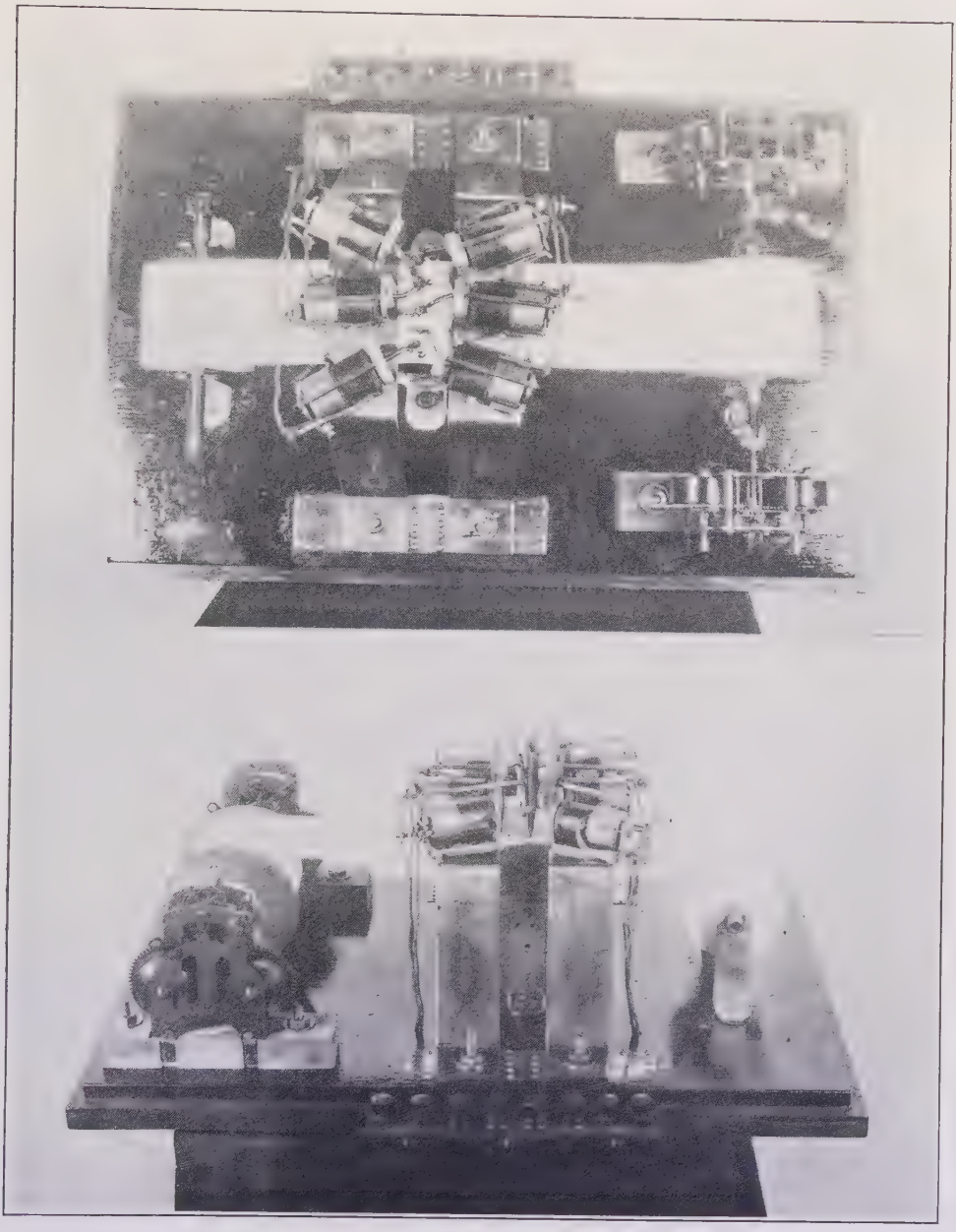


Fig. 11. Recording instrument with six circuits as seen from above and from one side. Photo by E. L. Caum.

of the beakers. A flat piece of rubber is cemented on the bottom of beaker "B" and the tube "D" is adjusted so that its lower end just touches, and is closed by, this rubber when the scale is balanced. Tube "C" is connected to the inlet tube "I" of the culture tank with rubber tubing. Tube "D" is extended with rubber and glass tubing, as indicated, so that it may serve as a siphon to withdraw water from beaker "B." To operate, beakers "A" and "B" are filled with water or culture solution and siphons are established from each through the tubes entering them

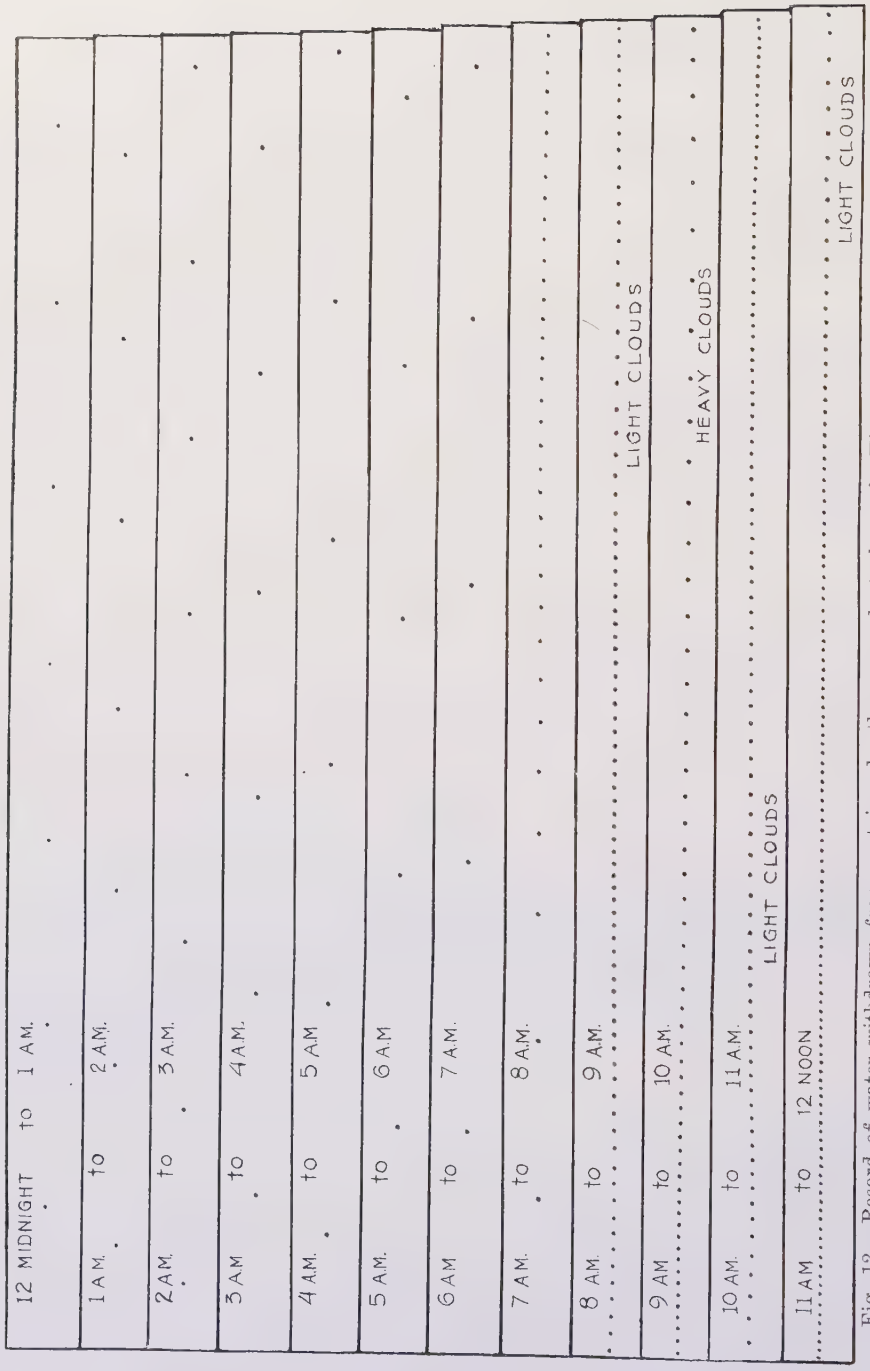

12 MIDNIGHT to 1 A.M.
1 A.M. to 2 A.M.
2 A.M. to 3 A.M.
3 A.M. to 4 A.M.
4 A.M. to 5 A.M.
5 A.M. to 6 A.M.
6 A.M. to 7 A.M.
7 A.M. to 8 A.M.
8 A.M. to 9 A.M.
LIGHT CLOUDS
9 A.M. to 10 A.M.
HEAVY CLOUDS
10 A.M. to 11 A.M.
LIGHT CLOUDS
11 A.M. to 12 NOON
LIGHT CLOUDS

Fig. 12. Record of water withdrawn from container by the cane plant shown in Fig. 4 during a period of twelve hours. Each dot records a trip of the scale, which was adjusted to weigh 4 grams.

from above. Water withdrawn from the culture tank by the cane plant will be replaced from beaker "A" through siphon "C." This will render beaker "A" lighter than beaker "B," hence "A" will rise and "B" will fall. The falling of beaker "B" will remove the rubber from the end of tube "D" and water will flow through this siphon until "B" rises sufficiently to close the opening of tube "D."

Thus, as the cane plant extracts water from beaker "A," an equal amount is automatically discharged from beaker "B."

When we designed this apparatus, we expected that the discharge through siphon "D" would be in periodic spurts, but in operation we find that the discharge is remarkably uniform. The tubes "C" and "D" affect the weight of "A" and "B" equal to that of the water which they displace and when beaker "A" rises, more of tube "C" and less of tube "D" are submerged and consequently these tubes act as a damper and temper the swing of the balance.

A trip-bucket scale, "E," is so placed that it will weigh the water discharged from siphon "D." This scale is adjustable so that it can be made to trip at any desired weight between 0.5 and 6.0 grams. The bucket discharges through a funnel into a container and the accumulated water is weighed to check the record of the scale. Every time the scale trips, it closes an electric circuit, causing a pencil to make a dot on a moving strip of paper.

In Fig. 7, the essential parts of our weighing apparatus are posed so as to show the arrangement of parts, but when in use, this apparatus is enclosed in a tight and rigid cabinet which is supported upon concrete posts, set deeply into the ground. Such a cabinet may be seen beside the cane plant in Fig. 4. The installation of the apparatus within the cabinet may be seen in Fig. 8. As beakers of equal size are used, the loss by evaporation is the same from each beaker, but as the entire apparatus is enclosed within a cabinet which is practically air-tight, the atmosphere soon becomes saturated, so evaporation does not cause an appreciable error in our results. The development of a satisfactory trip-bucket scale has required considerable experimentation. The one used for obtaining records from the plant shown in Fig. 4 is illustrated in Fig. 9. A model which we are now using is illustrated in Fig. 10. In our earlier models, the electric current was passed through the axle, but arcing injured the bearings and we have employed other channels for the circuit: in the instruments now in use, two platinum wires on the underside of the bucket dip into two mercury cups and close the circuit. The trip buckets are made of thin copper and, after fabrication, are electroplated with silver.

In the recording instrument employed in our experiments (Fig. 11), paper tape is rolled off from one drum onto another, passing in transit over a small block or table. Recording pencils are poised over this table and whenever current passes through a coil controlling a pencil, this pencil descends, making a dot on the paper tape. One recording pencil is employed to mark the time on the tape. A simple attachment on a clock serves to close a circuit at desired intervals and these intervals are indicated by dots on the tape. In our experiments, we are using 15-minute intervals, but any other interval desired may be as easily employed. A record of water consumption by the cane plant shown in Fig. 4 through a period of 12 hours is reproduced herewith as Fig. 12.

One important matter that must be considered in conducting experiments of the nature described above is the proper aeration of the water or culture medium about the root system of the plant. Continuous records of the water extracted from the sealed container by the cane plant shown in Fig. 4 were usually made through periods of 24 hours, although a few records obtained cover 48-hour periods. During the intervals when records were not being taken, a gentle flow of tap water was maintained through the container and this provided the necessary aeration.

Cane Diseases in Mauritius

By J. P. Martin

In the Annual Report of the Department of Agriculture of the Colony of Mauritius for the year 1928 there appeared several interesting paragraphs pertaining to sugar cane diseases. The salient points in these paragraphs may be briefly summarized as follows:

Leaf Scald

This disease was recorded for the first time during the year (1928) but undoubtedly had existed for many years and had been confused with gummosis or gumming disease of sugar cane. Leaf scald is now known to be widespread over the island. It attacks chiefly White Tanna and Striped Tanna canes and results in losses both in the field and factory. Experiments have been conducted wherein losses of from 5 to 11 per cent of cane weight occurred and the sucrose content reduced as much as 16 per cent. White Tanna is tolerant to the disease and fair yields are still being obtained under favorable climatic and cultural conditions. The disease affects not only the standing cane but greatly inhibits the germination of cuttings and the ratooning qualities.

The methods of control are the substitution of resistant varieties when feasible, the disinfection of cuttings (the method of disinfection is not given) and the selection of healthy cuttings for commercial propagation.

Gumming Disease

Gumming disease is similar to leaf scald in that it causes a loss of cane weight and sucrose content. It also seriously affects the germination of cuttings and retards the ratooning qualities of stools. No varieties are immune, but some offer a high degree of resistance to the disease. White Tanna is widely affected but, with this variety, the losses are less than they are in the case of leaf scald disease. M. (Mauritius) 55 and 131 manifest heavy leaf infection from gumming but little stem infection. D. K. 74 and R. P. 8 are highly resistant to both leaf scald and gumming. Two other local seedlings, M. 2316 and M. 2716, also exhibit a high degree of resistance to gumming disease.

Leaf scald and gumming exhibit somewhat similar leaf symptoms during certain periods of their development and are at times listed together during plantation surveys. Injuries from these two diseases were slightly less in 1928 than in 1927. In both diseases leaf infection causes much smaller losses than stem infection.

Measures recommended for the control of gumming are: the selection of healthy planting material, the establishment of nurseries with healthy cuttings from which a supply of healthy cuttings for plantations may be secured, and the replacement of susceptible varieties by more resistant ones. New seedlings that prove to be highly susceptible to gumming are immediately eliminated.

The results of preliminary expermients regarding the control of the two diseases by the so-called "hot water treatment" are interesting. In the majority of cases immersion of cuttings in water at 60° C. for 15 minutes killed the buds. Healthy cuttings of White Tanna and cuttings affected with leaf scald and with gumming were immersed in water at 50° C. for one hour and at 55° C. for one-half hour. Similar lots of cuttings were planted without the hot water treatment. The experiment is not sufficiently advanced to indicate definite conclusions, but in most cases the treatment for one hour at 50° C. stimulated germination. Both healthy and leaf-scald-affected cuttings withstood the treatment at 55° C. for one-half hour and showed a high percentage of germination. The cuttings affected with gumming disease and treated at 55° C. for one-half hour showed a low percentage of germination.

Red Rot

Of the varieties White Tanna, D. K. 74, M. 131 and M. 55, the D. K. 74 was most susceptible to red rot disease. White Tanna was highly resistant to red rot. A serious outbreak of red rot occurred on R. P. 6 on one estate. In certain localities the disease was severe but in general the losses in 1928 were less than in 1927.

Pineapple Disease

Losses from pineapple disease are manifested through the failure of cuttings to germinate or in the stunting of young shoots, especially in regions where dry weather generally prevails.

Smut

"Caused by *Ustilago scitaminea,* is still almost confined to the drier regions of the island."

Streak Disease

Streak disease is confined to R. P. 8 and was rarely encountered during the year. Losses from this disease were negligible.

Root Disease Complex

Instances of the root disease complex, the exact cause of which is still obscure, were reported from widely separated areas. A disease which produces a wilt and causes mature canes to die rapidly is at present included under the heading of root disease complex and seems to be parasitic in its origin.

Pokkah Boeng

The condition recognized as pokkah boeng in Java and in other cane-growing countries was recorded during the year. The condition seems to be associated in some rare cases with a heart or top rot. Losses from the disease were small.

Eye Spot

Heavy attacks of eye spot on leaves of White Tanna were encountered on a few estates during the wet season of April. No losses from eye spot disease were reported.

Stem Deterioration

This trouble is widespread, especially on White Tanna and Striped Tanna canes. It is manifested "by a dry, white, pithy deterioration in the center of the stem which later turns red to reddish-brown with yellow patches on either side of the affected stem. Later the center of the stem becomes hollow. The condition is not regarded at present as being parasitic in its origin. Bacteria have been isolated from affected tissues, but they were not proved to be parasitic." Further investigations are being conducted on this trouble.

The Cane-Killing Weed

A rather severe attack of *Striga hirsuta,* which is a flowering parasitic plant affecting sugar cane roots, was observed on one estate.

Cane affected with this parasitic weed is stunted and the whole plant has a withered appearance. The parasite may be found growing near the base of the affected stool.

Forestry on Oahu

By George A. McEldowney

This Unit was organized in 1919 under the supervision of the department of botany and forestry, to cooperate with the officers of the Board of Agriculture and Forestry in promoting forestry work on the island of Oahu. The results sought through the work to be undertaken by this Unit were to protect and increase the forest cover on the watersheds of Oahu so as to conserve the water falling thereon and divert it into useful channels.

Reconnaissance

The first work undertaken by this Unit was to gather all available information concerning the lands on this island, their boundaries, ownerships, topography and appropriate classification as forest lands, agricultural lands, grazing lands and waste lands. Much time was spent in consulting authorities, maps and land records, and all data obtained were checked in the field. A series of indexed maps was next prepared, setting forth this data in graphic form. These maps have proved invaluable to us in every phase of our later work. This is particularly true of the maps showing (1) boundaries, ownerships and leaseholds; (2) classification of lands according to their most appropriate uses; and (3) the nature of the cover on forested lands and the extent of lands that should be reforested.

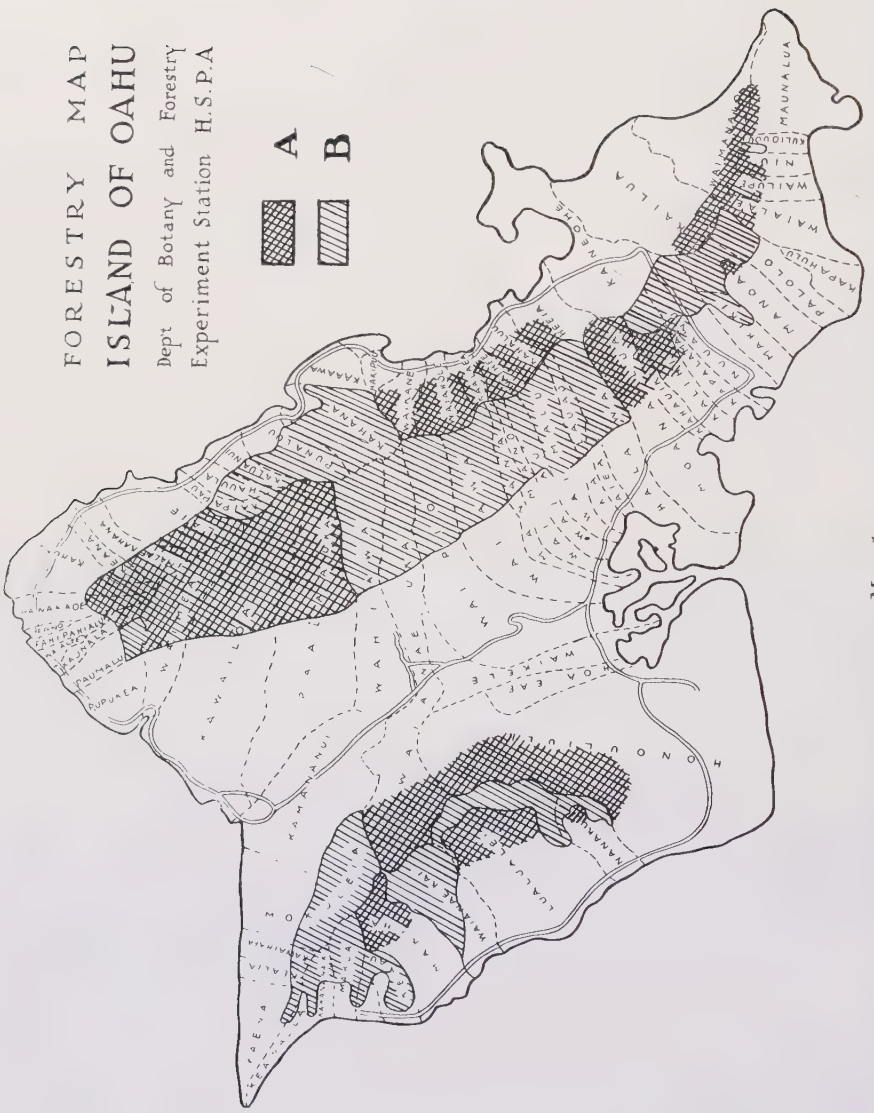
Forest Reserves Increased

When Unit No. 2 was organized in 1919, 67,333 acres of land on Oahu had been designated as "Forest Reserves" (areas shown in the light shading on Map 1). It was at once apparent that these reserves did not include some of the most important watershed areas on this island and our investigations showed that a very considerable part of these watershed areas not in reserves were being devastated by grazing animals; in fact, they were being heavily stocked as ranch lands and, being privately owned, were not subject to governmental control.

It was obvious that forestry work on Oahu would be more or less futile until additional large areas of watershed were set aside and protected as forest reserves. We presented these facts as clearly as possible and soon public sentiment was aroused to the extent that means were found for acquiring control of the land and putting it into forest reserves under the jurisdiction of the Board of Agriculture and Forestry.

Since the organization of Unit No. 2, 52,844 acres of land on Oahu have been added to the forest reserves (areas shown in dark shading on Map 1), making a total of 120,177 acres, or 31 per cent of the entire island. This, we believe, is pretty close to the maximum amount of land that can reasonably be set aside and administered as forest reserves on this island.

In the reorganization of the forest reserves of Oahu, it was necessary to run



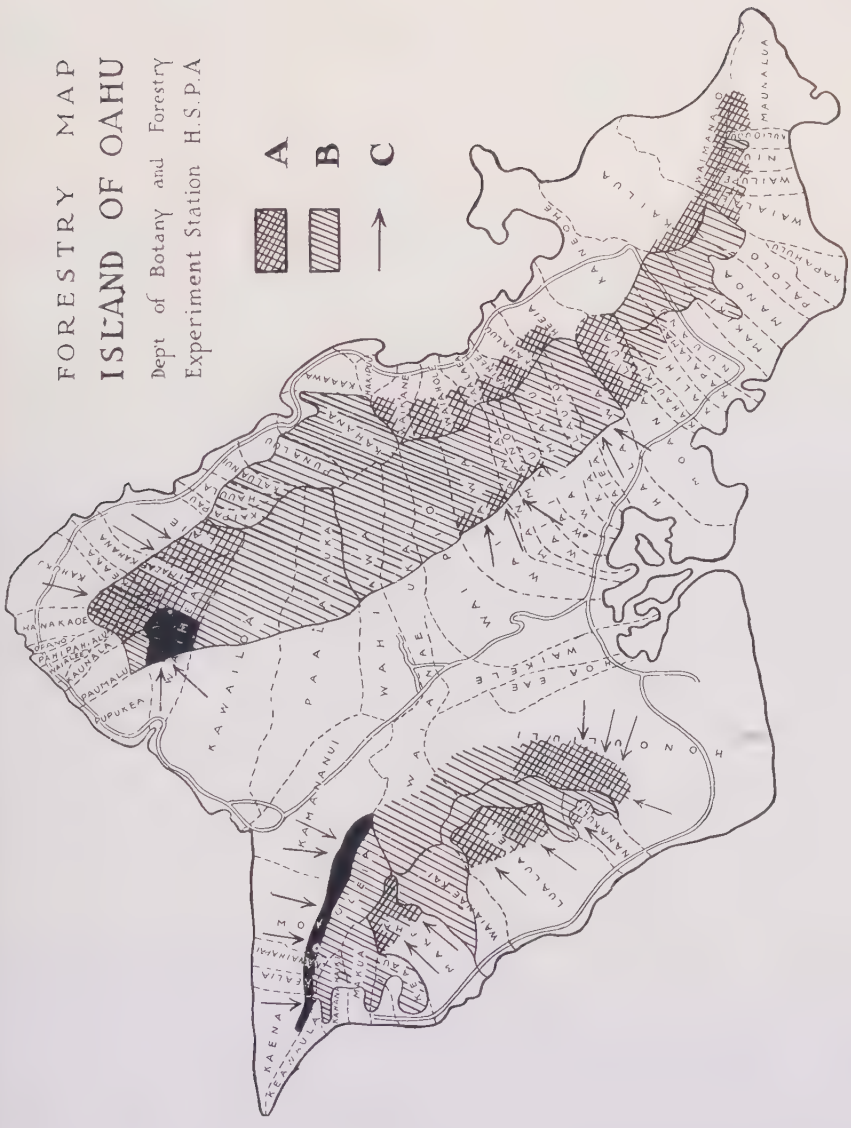
Map 1

A—Areas in Forest Reserves in 1919 ..... 67,333 acres
B—Areas added to Forest Reserves during past 10 years..... 52,844 acres

Total area in Forest Reserves in 1929..... 120,177 acres

boundaries in the field, not only around the new areas, but around all the old reserves, as many of the lines established in the earlier proclamations were not judiciously placed or were inadequately marked in the field. The running of these new boundary lines was done under the personal supervision of C. S. Judd. We accompanied him in the field and assisted in the marking of all courses. Our maps, previously prepared, proved of inestimable value in carrying out this work.

As soon as forest reserve boundaries were marked in the field and properly surveyed, the officers of the Board of Agriculture and Forestry undertook the construction of new fences and the rehabilitation of old fences along all boundary lines which were exposed to grazing animals. Some of these lines extended across very rugged country and presented serious obstacles for the fencers to overcome.



Map 2
A—Former ranch lands, now Forest Reserve, from which stock has been excluded during past 10 years.
B—Forest Reserve lands on which stock is still allowed to run.
C—Cattle adjacent to Reserve.

Great credit is due the Board of Agriculture and Forestry for the speed with which they completed very substantial fences of durable materials along all boundary lines where fences were required. Following the completion of the fences, the removal of roving stock was undertaken and we believe that, at this time, very few horses and cattle are to be found roaming within the forest reserves on this island.

Forest Plantings

At no time since its inception has this Unit been in position to make a record in tree planting. In fact, during the early years of its existence, we were instructed to go slow with planting operations and concentrate our energies upon reconnaissance, mapping and the collecting of data showing the necessity of placing such areas as

Honouliuli and Kahuku watersheds in forest reserves. Despite this handicap, Unit No. 2 has been responsible for the planting of 786,320 trees on Oahu since 1921. Some 503,000 of these trees have been planted in forest reserves or on watershed lands directly adjacent thereto. Plantations have planted about 157,000 trees on barren or waste lands outside the reserves for forest cover or commercial timber. Approximately 124,000 trees have been supplied for camp, park and roadside plantings.

The United States Army at Schofield Barracks has shown great interest in reforestation and has done some excellent work along this line, making very extensive plantings.

Most of the tree planting within forest reserves done by the Unit has been in the nature of experiments to determine suitable trees for employment under the conditions, and at the elevations, presented by the denuded areas within our foerst reserves. Ten years ago, eucalyptus and silver oak were about the only exotic trees known which could be grown to advantage within our forest reserves.

Forest Unit No. 2 made its first experimental planting in May, 1921. The area planted is located on the slopes of Kaukonahua Gulch above Wahiawa. All the varieties of trees then available in the Honolulu Nursery were planted in this experiment. The majority of these failed to make satisfactory growth under the conditions offered, but this experiment demonstrated that the paper-bark tree, *Melaleuca leucadendron;* the Formosan koa, *Acacia confusa;* and the Norfolk Island Pine, *Araucaria excelsa,* were admirably suited for planting within the forest reserves on Oahu. This experiment served to bring these trees to light for the first time and demonstrate their superior qualities. The conclusions reached from this experiment have been verified by later plantings and these three trees are bound to become important components of the future forests of this island.

The production of groves of fig trees at intervals within our forest reserves has been a definite part of our plan throughout all our forest plantings. We have obtained good stands of *Ficus macrophylla, Ficus rubiginosa, Ficus glomerata* and *Ficus nota.* We have shown photographs of some of these in our former reports, but the trees have now grown so large and mingled to such an extent with the native vegetation that it would be difficult to show them in photographs unless we made extensive clearings.

The wasps associated with the two species of figs first named above are now found on all parts of the island where these trees occur. These tiny wasps have made their way, unaided, all the way to Laie from Honolulu and always appear to be on hand at the first fruiting of a new tree. Results attained through the scattering of the seed of these trees by birds which have eaten the fruit should be noted within the next few years.

Planting Seeds

We have obtained very good results from planting seeds directly in the ground. We have employed the seeds of many different species of trees in experiments of this sort and believe that in the case of some species, we obtain better results from seed-spotting in the field than we do from setting out seedlings which were grown in the nursery. This is particularly true of trees that produce a tap root.

We have also obtained good results from *Ficus* seed thrown by hand onto stumps and logs along the trails within the forest. The favorable results obtained from seed spotting and broadcasting has led to the sowing of seeds from airplanes. The employment of this method of seed distribution has been made possible through the cooperation of the United States Army. On numerous occasions, they have supplied the airplane to distribute seeds over areas of our forest reserves which we have designated. In recent months, the Air Service, U. S. Army, at Wheeler Field, has made eleven trips into the air with its large Fokker plane in order to scatter quantities of tree seed for us.

We are often asked if any trees have grown as the result of this airplane sowing. We have always answered this question cautiously. We have numerous trees now growing from seed which was scattered by hand along forest trails. When we find a tree growing in the vicinity of a forest trail, we cannot be sure whether it is from seed sown from an airplane or from the trail by hand. It would be a difficult matter to locate one of these trees from a trail and a good deal of our airplane sowing has been done over areas not easily reached from a trail. One should consider that an airplane flying 800 feet or more above the ground, traveling 100 miles an hour, with its propellers whirling, must scatter the seed far and wide. It would be difficult to locate the seeds that sprout in a rough, partly wooded country unless one happened to stumble directly upon them. If, at the same time we made our first sowings from an airplane, fig seeds had been planted in the open and given the best of care, the resulting trees would not now be over 10 feet tall. A tree of that size could not be easily spotted at any considerable distance in our forests. We do not expect every seed we scatter to grow. Nature does not achieve that, so the trees resulting from our airplane sowings would probably be few and far between in any given area. They should suffice, however, to infect the locality with the tree in question and produce a focus from which it would later spread.

In recent months, we have found a fine specimen of *Ficus macrophylla* growing on an old koa stump in a deep gulch, far away from any forest trail, but in an area covered by our first sowing from an airplane. There can be little doubt but that this tree is a result of airplane seeding.

Arboretums

The Unit maintains two charted arboretums, one of 15 acres in the old townsite park at Wahiawa, which is now a forest reserve; and the other at Poamoho, which is also in the Wahiawa district. This latter arboretum is at 1,400 feet elevation and now includes about 20 acres, but can be extended at will, as it is inside the forest reserve. In this arboretum, we test out all trees available for forest planting under actual forest conditions. If they make favorable growth here, we plant them elsewhere within the reserves, but if they do not grow, we take them out and plant other trees in their stead. At this arboretum, we now have thirty varieties of our latest introductions under observation.

Wahiawa Nursery

Unit No. 2 organized a forest tree nursery some six years ago at Wahiawa. This nursery now covers 5 acres of land in the old townsite park, adjacent to our

Wahiawa Arboretum. We use this land, rent free, and the Wahiawa Water Company supplies us with water, gratis. The soil is excellent and the situation is ideal for our purpose. The elevation is 1,100 feet and the seedlings of many forest trees grow better here than they do in the nursery at Honolulu.

In addition to serving as a suitable place for growing tree seedlings, this nursery affords storage space in which seedlings grown at the Honolulu Nursery are held, sorted and distributed to various points on the island for planting. Since its opening, there has been distributed from this nursery an average of 800 trees a month. One man makes his headquarters at the Wahiawa Nursery but attends to planting details at the Poamoho Arboretum and at other points within the forest reserve in addition to looking after the nursery stock and the distribution of trees.

Plantings on Plantation Lands

We have frequent calls from plantations for assistance in making camp, park and roadside plantings. To meet this demand, we grow and supply a large variety of shade, ornamental, fruit and nut trees. Although this is not strictly forestry work, it is, nevertheless, an important function of the Unit and one which we are pleased to perform to the best of our ability.

Several plantations have made rather extensive plantings of trees on their waste lands with the idea that these may be used at some future time for fence posts, firewood, railroad ties, etc. One plantation now has a 500-acre forest of eucalyptus trees including many of the better-known species. These trees were planted out only 16 years ago but the plantation is now taking from this grove each year some 1,200 cords of firewood, 100 cords of ditch posts, 87 cords of fence posts and 8 cords of car stakes. As yet, they are harvesting only *Eucalyptus robusta* and *Eucalyptus globulus*. Some of the better varieties, when full-grown, should yield very valuable timber. One of the public service corporations in these islands now sends to the mainland $25,000 a year for poles alone. It has been demonstrated that poles, to meet their requirements, can be grown in these islands in from 15 to 20 years.

Wild Animals in Forest Reserves

As noted in a previous paragraph, roaming cattle and horses have been practically eliminated from the forest reserves on Oahu through the efforts of the Board of Agriculture and Forestry. It is now difficult to find traces of cattle at any points within the reserves along the southern slopes of the Koolau Range. On the windward side of the Koolau Range and in some parts of the Waianae Mountains, cattle still invade areas that should be protected. Wild goats are now making their last stand in inaccessible parts of the Waianae Range. They are so closely and constantly watched by hunters that they will never be able to multiply to such an extent as to become a menace to our forests. Wild cattle and goats have been brought under satisfactory control but the wild hog had increased to such an extent that it is as serious a menace to our forests as either of the other animals has ever been. They eat seed and young seedlings, preventing natural reforestation, and they root up, and destroy, ferns and all other types of undergrowth.

They dig up the soil over large areas and rapid water erosion follows. The damage which they are causing is enormous and it is most serious because it is in the very interior of our forest reserves.

Many pigs are being killed by hunters, but the operations of the hunters simply drive the majority of the pigs into the recesses of the forest where the hunters will not pursue them. Wild pigs will never be eliminated from Oahu forest reserves by casual hunters who pursue them merely for sport or the meat which they supply. Our experiments in trapping pigs have given encouraging results and we believe this method of procedure offers the greatest promise of bringing about their ultimate eradication. The control of wild pigs is one of the most serious forestry problems of Oahu.

Firebreaks

The uluhi fern, which extends in an almost unbroken blanket throughout the length of the Koolau Range in the makai portions of the forest reserves, presents a fire menace that should receive serious attention. Should a fire get started in this fern during one of our periodic dry spells, it would be apt to travel far and do great damage before it could be brought under control with conditions as they are at the present time.

We believe that insurance against such a catastrophe should be obtained by building firebreaks through this fern blanket from makai, mauka along some of the prominent ridges. These firebreaks would prove a satisfactory line of defense against any fire that might get started and they would also afford an easily traveled avenue along which men could be moved to get at any fire that might get started in the interior of a forest reserve.

The great value of a firebreak has been demonstrated on several occasions by the very efficient one which has been built and maintained by the U. S. Army, at our suggestion, on the Waianae Range back of Schofield Barracks. It has not only stopped fires but has served as a trail along which fire fighters could quickly get to any danger point. We believe that the building of several firebreaks on the Koolau Range by Unit No. 2 would be a very valuable contribution to the cause of forestry on Oahu.

Control of Erosion

There are large areas of land within, or along the margins of, forest reserves in which we are particularly interested on which serious and rapid erosion is taking place. Heavy stocking and temporary cultivation are the factors which started the erosion in most cases and with the surface of the soil once broken, rain and wind are rapidly eating it away, leaving enormous earth scars, which are constantly growing in size and ugliness.

We have undertaken extensive experiments designed to find a means of stopping erosion and covering the scars with vegetation. Some degree of success has been obtained in several ventures, but we believe that a coarse grass which will act as a binder is going to prove the best plant to use as the first stage in this reclamation work.

Rain Gauges

In studying the rainfall records of Oahu and the data on which these records were based, it became evident that authentic readings must be made at additional stations before one could arrive at an approximately accurate estimate of the annual rainfall of this island.

An inventory of the existing rain gauges was made and it developed that the gauges were being read at ninety stations on the watersheds surrounding the Pearl Harbor Basin. The locations of the existing stations were plotted on a map and it was found that at least twelve additional stations must be maintained before we would be able to gather the required data. Up to the present time, gauges have been installed by Unit No. 2 at eleven of these stations and readings are taken at regular intervals. The installation of these gauges and their readings has required the building and maintenance of many miles of trail. It takes 12 hours of hard tramping to get a reading from one of these gauges. Our gauges are made of copper, according to specifications supplied by the Honolulu Sewer and Water Commission, and they record up to 26 inches of rainfall.

Through the cooperation of the Honolulu Plantation Company, Waiahole Water Company, Oahu Sugar Company, Waialua Agricultural Company and the Hawaiian Pineapple Company, readings of these gauges are made at regular intervals by trained observers. Records are kept on file at our office in Honolulu and are also supplied to the office of the U. S. Weather Bureau in Honolulu.

Future Work of Unit No. 2

There is considerable public sentiment, which has found expression in several ways, that this Unit should confine its attention to problems which must be solved by experiment and leave the routine planting of trees within the forest reserves to the Board of Agriculture and Forestry. This would seem to be an appropriate arrangement, as the Board has ample funds to do as much tree planting as expediency requires.

We believe, however, that the Unit should continue work along all of the lines in which it is now engaged. These may be enumerated as follows:

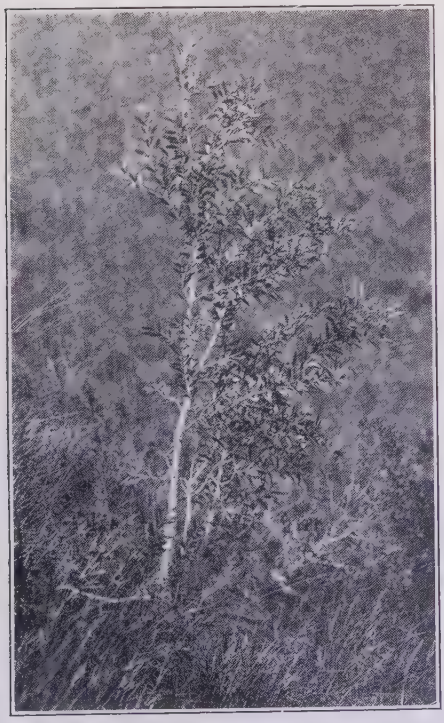
1. Maintaining a forest nursery at Wahiawa for the distribution of trees.
2. Testing new trees in arboretums for the detection of desirable species.
3. Making small experimental plantings at intervals throughout the forest reserves in which we are most interested.
4. Conducting experiments in the control of wild pigs.
5. Conducting experiments in the control of soil erosion.
6. Securing and recording rainfall data.
7. Cooperating with plantations in the planting of commercial timber.
8. Cooperating with plantations in making camp, park, roadside and waste-land plantings.
9. Cooperating with the U. S. Army in making plantings on Army lands.
10. Cooperating with the Air Service of the U. S. Army in the planting of tree seeds from airplanes.

If the Board of Agriculture and Forestry will sanction the construction of firebreaks, we believe that the Unit should undertake this work as a new project. To build an appropriate firebreak, a trail should first be constructed along the crest of a ridge. The uluhi fern should then be cut away for a considerable distance on either side of this trail and the cleared space planted up with non-inflammable trees and shrubs. Operations of this sort would supply not only protection against the spread of fire, but would build lines of exotic plants through our decadent forests and these plants would eventually serve as sources for the spread of the constituent species.

Ficus macrophylla, Moreton Bay fig. Growing on koa tree-stump from seed scattered by airplane in 1923.

Araucaria excelsa, Norfolk Island pine, 8 years old, at Wahiawa. Planted May, 1921; photographed August, 1929.

Melaleuca leucadendron, Paper-bark tree, 8 years old. Planted in 1921; photographed in 1929.

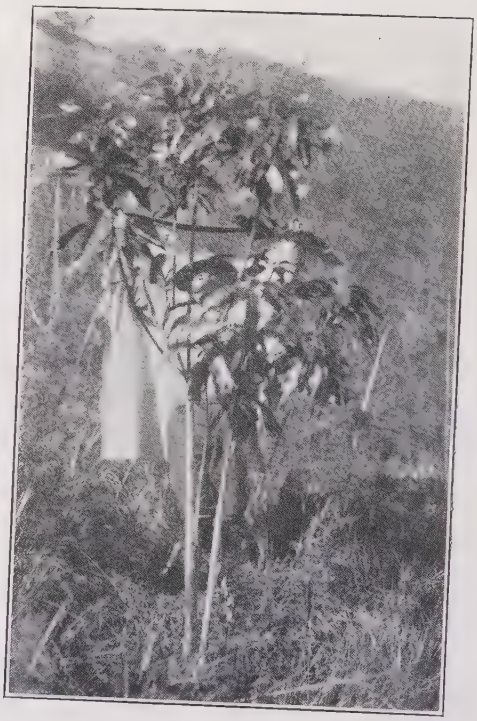


Paper-bark tree, 2 years old. From seed spotting.

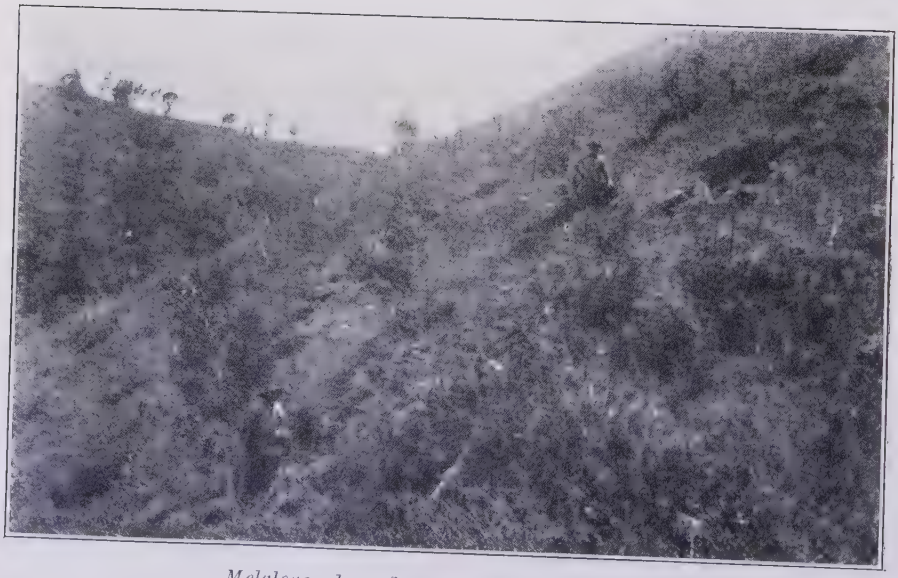
Paper-bark tree, 2 years old. Planted on waste land.

Some results from Seed Spotting in 1927.
Photographs taken in 1929.

Rose Apple



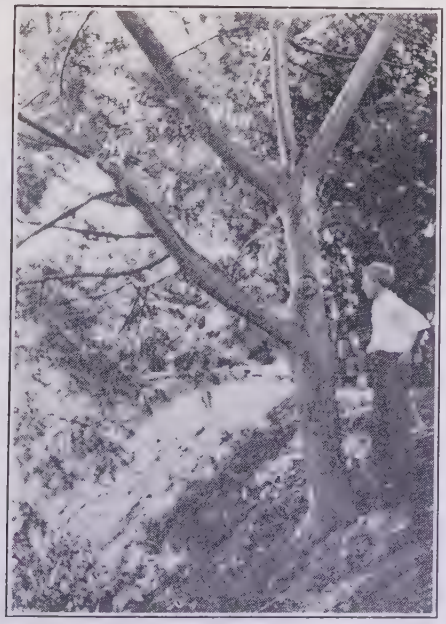
Mango



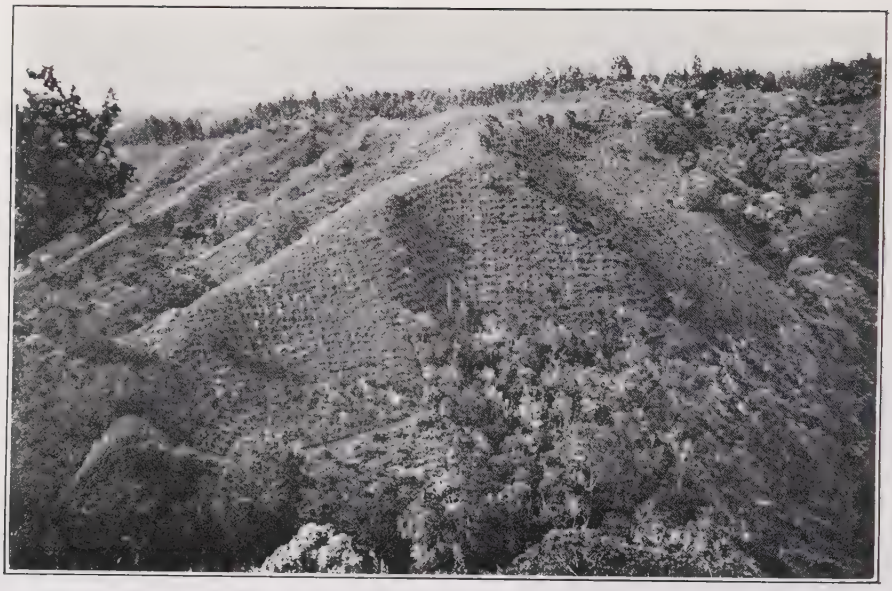
Melaleuca leucadendron, Paper-bark tree.

Ficus nota, Wild fig.

Terminalia myriocarpa, "Jhalna," 8 years old. At Oahu Sugar Company.



Trema orientalis, "Charcoal tree," 4 years old.



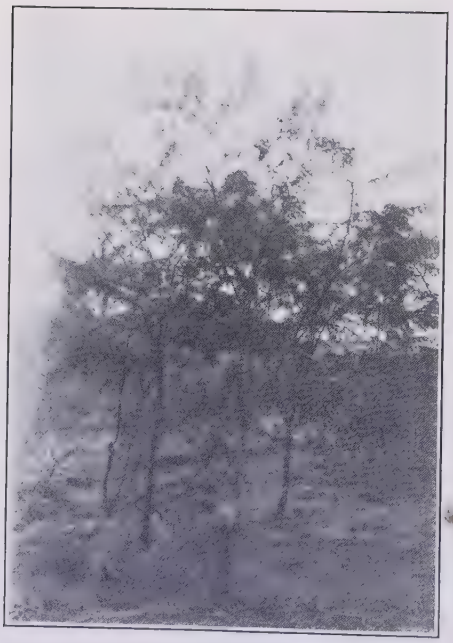
Forest reserve planting at Waipio. John Ii Estate.

Casuarina equisetifolia, Ironwood. Planted along waterways to keep down grass growth. Kahuku Plantation Company.

Extensive Waste-Land Planting
by Oahu Sugar Company.

Waste-land Plantings
by Honolulu Plantation Company.
Planted in 1927. Photographed in 1929.

Albizzia moluccana, 2 years old.

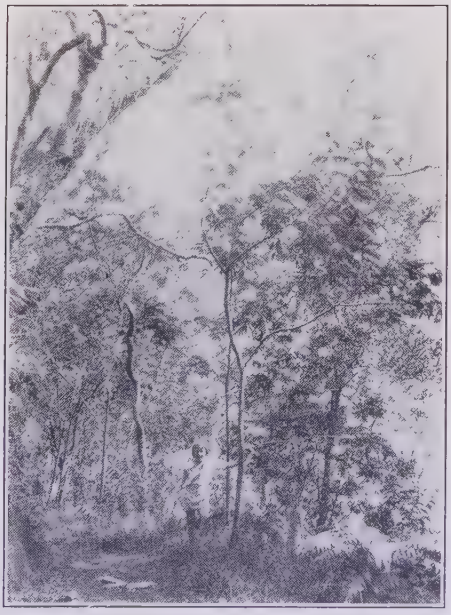


Acacia arabica.

Albizzia moluccana, 2 years old.

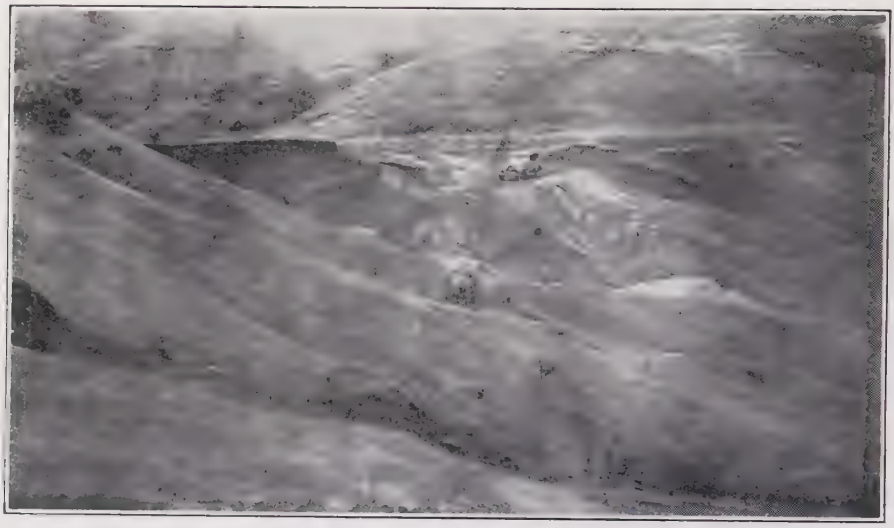
Site of Poamoho arboretum as seen from the air.

Burning *Uluhi*, staghorn fern, in clearing for Poamoho arboretum.



Albizzia moluccana, 2 years old. From seed spotting.

Types of Erosion we are Trying to Control.



Firebreak, 50 feet wide. Built by U. S. Army, Schofield Barracks.

Ornamental planting, using *Ficus rubiginosa* trimmed to shape. At Ewa Plantation Company.

Cedrela australis, 3 years old. At Waialua Agricultural Company.

Gate post, 25 years old. *Eucalyptus globulus*. At Maunawili Ranch.

View from air of waste-land planting. Waialua Agricultural Company. Photographed in 1928 by Eleventh Photo Section, U. S. Army Air Service.

U. S. Army Fokker airplane loading bags of tree seed.

Some Observations on the Wilting Coefficient of a Selected Waipio Soil

By H. A. Wadsworth* and U. K. Das†

The purpose of the investigation reported in the present paper was threefold:

(1) A preliminary study of the physical properties of the selected soil and the determination of its critical soil moisture constants. Proposed work on the water cost of sugar cane and of sugar under Hawaiian conditions required a more complete knowledge of the properties of the selected soil type than was available.

(2) To determine whether the highly colloidal soil under investigation possessed a more or less definite wilting coefficient as defined by Briggs and Shantz(4).

(3) To determine the mathematical relation between this wilting coefficient, if one is determined, and other measurable soil moisture constants, such as the moisture equivalent and the maximum water-holding capacity.

Briggs and Shantz(4) define the wilting coefficient as the moisture content of the soil (expressed as a percentage of the dry weight), at the time when the leaves of a plant growing in that soil first undergo a permanent reduction in their moisture content as a result of deficiency of soil-moisture supply. By permanent reduction is meant a condition from which the leaves cannot recover in an approximately saturated atmosphere without the addition of water to the soil. These investigators working with more than a score of soils and hundreds of species, report results which lead them to conclude that the wilting coefficient is a definite soil moisture constant. Slight differences in residual moisture which occur when different species are used for indicator plants are attributed to a more perfect root distribution with one species as compared with another and not to the ability of one variety to exert a greater attractive force upon the soil moisture than another.

A mathematical analysis of the results obtained from these experiments in comparison with other commonly used soil moisture constants suggested the following equations:

$$(1)\quad \text{Wilting coefficient} = \frac{\text{Moisture equivalent of Briggs and McLane(3)}}{1.84\ (1 \pm 0.007)}$$

$$(2)\quad \text{Wilting coefficient} = \frac{\text{Hygroscopic coefficient of Hilgard}}{0.68\ (1 \pm 0.012)}$$

Although the second of these formulas has been discredited by Puri(7) and others who object to the use of the hygroscopic coefficient as a critical moisture constant, the first remains as a useful tool in irrigation research.

Although Veihemeyer and Hendrickson(10) have verified the findings of Briggs and Shantz with respect to the insignificance of botanical differences in indicator plants used for the determination of the wilting coefficient, these in-

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vestigators report a greater variation in the constant factor in the denominator than is indicated by Briggs and Shantz. In fact, a value as high as 2.27 is reported for Stockton clay which, according to the U. S. Bureau of Soils, carries about 60 per cent colloidal clay. There is no known relation between these empirical constants and the type of soil used. Apparently there is no method of determining this ratio except by an actual trial with the use of plants.

Not all investigators will grant that the residual moisture in a soil at the time of true wilt depends entirely upon the soil type. Caldwell(5) and Shive and Livingston(8) seem to find that the wilting coefficient is dependent upon the intensity of the evaporating power of the environment for the period during which permanent wilting is attained.

Most research workers in irrigation believe the results of Briggs and Shantz to be sound in principle at least.

Since the wilting coefficient represents the lower limit of readily available soil-moisture its application to irrigation studies is evident.

Physical Properties of the Soil Used

As has been indicated the soil used for the observations reported in this paper was secured from the experimental field of the H. S. P. A. substation at Waipio. The site selected was typical of a rather large area. The soil was fairly uniform to a depth of three feet and well drained. It was doubtlessly alluvial in origin.

A small area, close to the selected site, was levelled, enclosed in a low dyke and flooded to a depth of five and one-half inches for the determination of the maximum water-holding capacity of the soil. An average of eight samples taken twenty-four hours after irrigation indicated a maximum water-holding capacity of 34.3 per cent. The variations between samples was so great, however, that the probable error in these results is high.

The moisture equivalents* determined from the same samples gave a value of 32.1 $\pm$ 0.2.

The colloidal content and fractional distribution of particles below arbitrary size limits was determined by means of a special hydrometer designed for this purpose by Bouyoucos(2). Volume weight determinations were made in the field by the use of viscous fluids as described by Beckett(1). A summary of these results, together with those already reported, is given in the following table:

PHYSICAL PROPERTIES OF WAIPIO SOIL USED IN WILTING COEFFICIENT DETERMINATION

Maximum water-holding capacity (average of 8 samples)	34.3 per cent
Moisture equivalent (10 samples)	32.1 $\pm$ 0.2 per cent
Real specific gravity (5 samples)	2.60

* The authors are indebted to K. B. Tester, of McBryde Sugar Company, Ltd., for the determination of the moisture equivalent, the colloidal content and the distribution of soil separates as shown.

Colloidal content (average of 10 samples)	51.2 per cent
Approximate per cent of particles below (average of 10 samples)	
.025 mm.	68.0 per cent
.010 mm.	54.0 per cent
.006 mm.	44.0 per cent
Volume weight (average of 6 samples)	1.1

Soil for use in the pots was secured from a shallow trench close to the flooded area. This material was mechanically screened in Honolulu and turned six times on a concrete floor to insure thorough mixing.

The pots used were gallon cans such as are used in canning plants. They were coated inside with a mixture of cement and sand and brought to a uniform weight of one pound three ounces by means of iron scrap placed in the bottom. Each can was filled to contain 6.65 pounds of oven dry soil, the moisture content of the mixed soil being determined by samples taken during the filling.

Thirty-nine cans were used. These were divided into four series. Three buff bean (*Tethonia speciosa*) seedlings were transplanted into each of twelve cans; three sunflower seedlings (*Helianthus annuus*) were transplanted to each of twelve cans in the second series, while each of the twelve cans of the third series was planted with a single eye, one joint, seed piece of sugar cane (H 109). Each joint weighed two ounces. Each of the remaining cans was planted with one bean, one sunflower and one cane eye.

The cans were carefully sealed to prevent loss of water by evaporation from the exposed soil surface. This was done by fitting a lead-foil disk into the can and waxing it to the sides. Small holes were provided for the stems of the seedlings. A general view of the equipment is shown in Fig. 1.

Since the tare weight of the can was known as well as the dry weight of the soil, the moisture content of any can could be determined by weighing the system. These weighings were made daily (Fig. 2).

The Determination of Wilt

As has been noted, true wilt is evident when a plant showing loss of turgor fails to revive when placed in a saturated atmosphere and a low temperature. If the plants regain turgidity, Briggs and Shantz assume that apparent wilt is due to a rate of transpiration in excess of the rate at which water is available from the soil. Such a condition might be occasioned by environmental factors making for a high transpiration rate, or a low conducting capacity in the plant tissue. Wilt of this sort is called temporary or incipient wilt. Such wilt may occur at any moisture content and can only be distinguished from permanent wilt by subjecting the plant to such an environment that the transpiration rate is greatly reduced. In the present experiment such an environment was provided in a high humidity chamber. This was a small, portable, cloth-walled closet placed in a tent which was constantly sprayed on the inside by fine streams of water. Dry and wet bulb

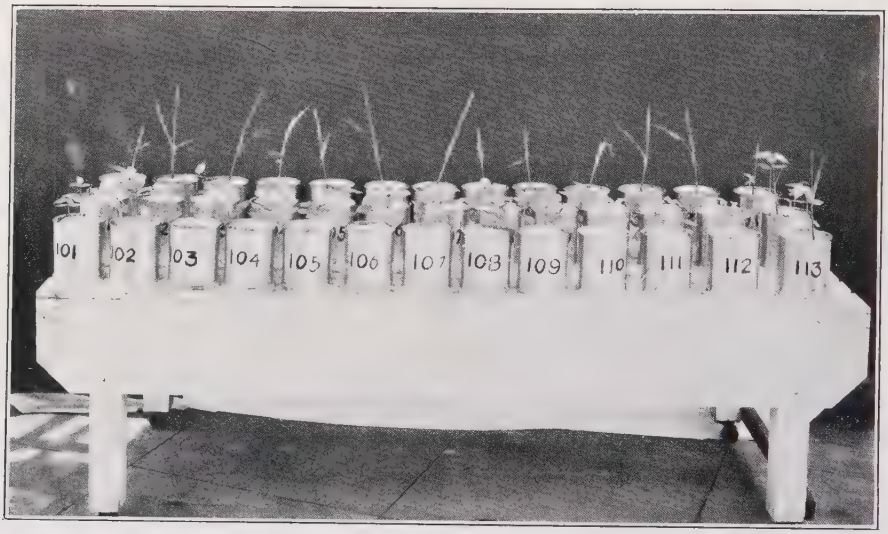

Fig. 1. A general view of the equipment.

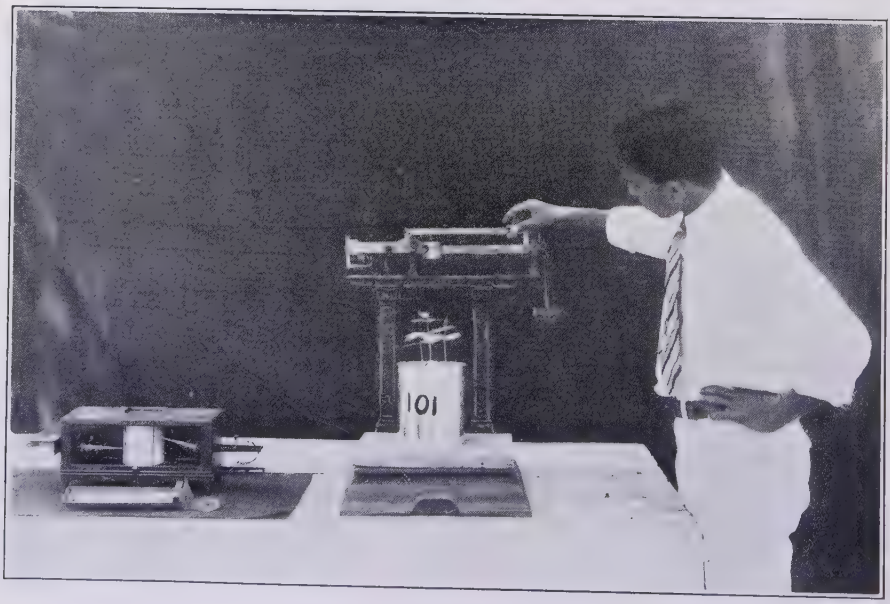

Fig. 2.

thermometers inside the chamber indicated a fairly constant temperature of 74° F. with a relative humidity of from 94 per cent to 96 per cent.

Plants showing signs of wilt were at once removed from the bench and placed in the high humidity chamber. If, after ten hours, signs of wilt persisted the wilt was called true wilt and the residual soil moisture content determined by careful weighing and computation. If complete turgidity was restored the plants were returned to the bench and the observations continued.

Recognition of Wilt

Perhaps the greatest source of error lay in correctly judging true wilt in accordance with the definition given above. As Briggs and Shantz(4) point out, transpiration continues long after this condition exists if environmental factors create a sufficient vapor pressure deficit about the transpiring surfaces. For this reason delay in removing a plant at the first sign of incipient wilt may result in error; if this is the only error involved the reported wilting coefficient is less than the real value.

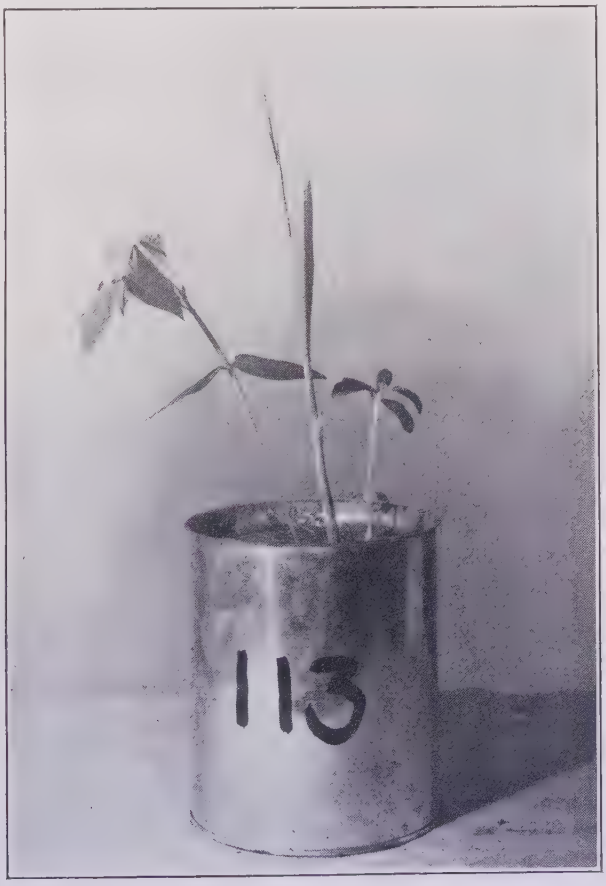

Fig. 3. Simultaneous wilting of three species. The three species show characteristic signs of wilt.

The plants used showed signs of wilt in different ways. The sunflower showed a slight curling at the edges of the leaves, the leaves becoming slightly "leathery" to the touch. The beans turned slightly darker in color; the leaves changed from concave, as viewed from above, to convex; the angle between the petiole and stem became sharply acute. Cane leaves showed a distinct curling along the axis. Since new leaves unroll upon normal development it is at times difficult to determine whether observed curl is due to a maturing of the tissue of a new leaf or due to wilt. Fig. 3 shows can No. 113 at the wilting point. Characteristic wilt is observed in all species.

Results

Bean Series: Soils in this series were allowed to reach the wilting coefficient twice. This was accomplished by removing the lead-foil seal from each can as wilt was determined and irrigating to maximum water-holding capacity. The seal was then replaced and the observations continued. The cans were opened at the time of the second wilt and the residual soil moisture determined by oven drying as a check on the methods and observations at the time of filling. Inspection of the root distribution at this time indicated an adequate distribution of roots within the soil mass.

The methods described above gave the following mean values with the corresponding probable errors:

First wilting	$23.3 \pm 0.14\%$
Second wilting	$23.5 \pm 0.10\%$
Residual moisture by oven drying	$23.7 \pm 0.10\%$

Fig. 4. Can No. 202 open for inspection.

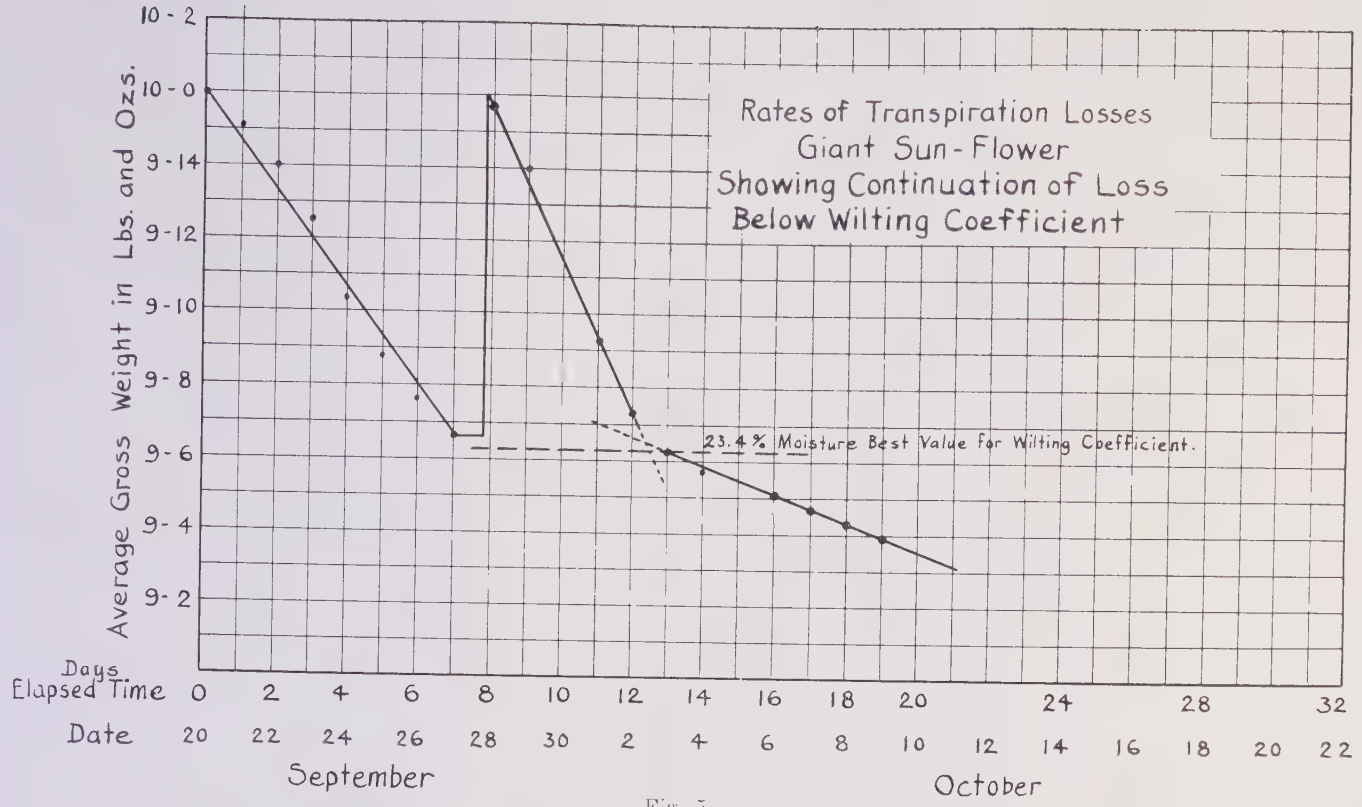
Rates of Transpiration Losses
Giant Sun-Flower
Showing Continuation of Loss
Below Wilting Coefficient
23.4% Moisture Best Value for Wilting Coefficient.
Average Gross Weight in Lbs. and Ozs.
10-2
10-0
9-14
9-12
9-10
9-8
9-6
9-4
9-2
Days Elapsed Time
0 2 4 6 8 10 12 14 16 18 20 24 28 32
Date
20 22 24 26 28 30 2 4 6 8 10 12 14 16 18 20 22
September
October

Fig. 5.

Sunflower Series: This series was allowed to wilt twice except for cans Nos. 202 and 205, which were opened at the time of the first wilt to determine the adequacy of the root spread and the uniformity of soil moisture depletion. Fig. 4 shows can No. 202 open for inspection.

Cans in this series were not destroyed for oven drying at the time of the second wilting, but were irrigated again and allowed to remain on the bench until death occurred; the weighings continued daily. The mean daily weight is plotted against time in Fig. 5. The change in rate of loss after the third wilting apparently indicates a change in the availability of soil moisture at a soil moisture content approximately equal to the wilting coefficient as determined by other means. From the uniform rate of loss between irrigation and wilting coefficient as is evidenced by the straight lines between September 20 and September 27, and between September 28 and October 2, one may conclude that water is equally available between these limits. In fact, Veihemeyer(9) concludes from similar curves and other evidence that "there appears to be no reason, either from physical considerations of the forces involved between the moisture and the soil particles or from physiological requirements of the plant, why optimum moisture conditions for growth should not vary from the maximum field capacity to about the wilting coefficient."

The results of the determination of the wilting coefficient by the use of the sunflower series are:

First wilting	22.8 ± 0.14
Second wilting	23.5 ± 0.16

Cane Series: Plants in this series showed physiological characteristics never before reported by investigators of the wilting coefficient. The logic upon which the method used with the bean and sunflower series is based assumes that there can be no absorption of water or water vapor by the leaves of a plant showing signs of incipient wilt, when that plant is placed in a saturated atmosphere. It is assumed that turgidity is restored by a movement of moisture from the soil, this rate of upward movement being great enough to exceed the reduced rate of transpiration in the high humidity chamber and to restore complete turgidity in the leaf tissue within something less than twelve hours. The present work seems to show that the wilted cane leaf is peculiar in that it possesses a decided capacity for absorption of water vapor or liquid water. A wilted cane cut at the ground surface and sealed at its cut end with wax regained full turgidity over night when suspended under a fine spray; a mature, discarded cane plant with wilting so complete that burning of the leaf tips had begun regained turgidity over night under a similar spray; plants in the cane series regained turgidity in the standard humidity house, although the soil moisture in the cane had dropped to 12.8 per cent because of transpiration through flaccid tissue as described under the discussion of the sunflower series. Although growth was handicapped or stopped entirely during this period it was impossible to note the time and consequently the residual soil moisture at the time normal growth ceased.

Additional evidence as to the water absorptive capacity of sugar cane leaves is furnished by the usual Hawaiian field practice of treating chloroitc plants with a

solution of iron sulphate, as reported by Verret(11). The cure is local initially. Dates and initials painted on chlorotic leaves appear in green upon a yellow background. Although the physiology involved is not clearly understood the cures may involve the admission of the solutions through the leaves. A dry dusting of the leaves of chlorotic plants with powdered iron sulphate seems somewhat less effective than solutions, according to observations at the Experiment Station, H. S. P. A. Lee and McHargue(6), working with the Pahala blight of cane, report little or no effect with dry iron sulphate dust and only a slight response with powdered manganese salts. It has been suggested that such material is only available as it is dissolved in water of condensation.

Because of this difficulty the usual method for the determination of the wilting coefficient with cane as an indicator was necessarily abandoned. All cans in the cane series were irrigated to maximum field capacity, giving gross weights of 10 pounds 2 ounces. The cans were weighed daily at 1:00 p. m. The results of these weighing are shown graphically in Fig. 6. The significant change in rate of loss at 9 pounds 8.3 ounces may indicate a change in the availability of moisture at 23.4 per cent, which corresponds to this gross weight. The nearness with which this figure approximates the results of other more standard methods of determining the wilting coefficient for this same soil type adds weight to such an assumption. Another factor contributing to this change in rate of transpiration may be curling of the leaves with loss of turgidity and a consequent protection of the transpiring structures. In any event there seems to be a critical soil-moisture content with cane, as in other plants, below which plants function differently than when soil-moisture is more abundant. And this critical soil moisture constant for cane is numerically close to the wilting coefficient for other plants. Apparently, if the term "wilting coefficient" is to be applied to soils of interest to sugar cane growers, the term must be redefined or used with reservation.

It must be borne in mind that other factors than unavailable soil moisture may have caused the results shown in Fig. 6. An abrupt change of environmental factors on October 25, which would reduce the evaporating power of the air and consequently lower the transpiration rate would cause a similar break. A recording thermo-hygrometer in constant operation showed no significant change in temperature or relative humidity at this point. In fact, the higher than normal temperature and lower humidity on October 27 would suggest a greater loss than usual on this day. No means were available for measuring sun intensities during this period.

These facts together with the distinct curling of leaves a few hours after sunrise after October 26, a slight retardation in rate of growth after this date and the close coincidence of the residual moisture content at this point with the standard wilting coefficient as determined with other crops, indicate that this change of rate of loss is of physiological significance.*

* Work subsequent to that reported in the main body of the paper indicates that the residual soil-moisture content at the time cane ceases growth is the moisture content which would result in wilt with other species.

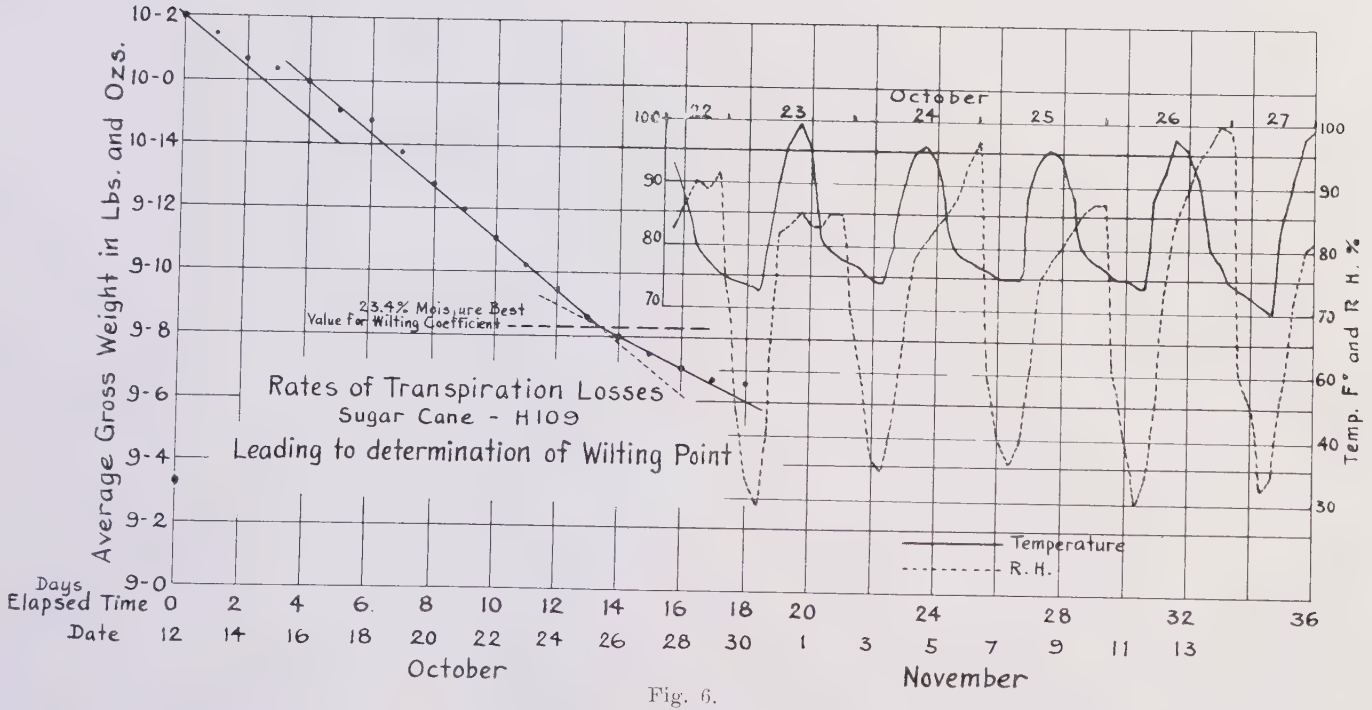
Average Gross Weight in Lbs. and Ozs.
10-2
10-0
10-14
9-12
9-10
9-8
9-6
9-4
9-2
9-0
23.4% Moisture Best Value for Wilting Coefficient
Rates of Transpiration Losses
Sugar Cane - H109
Leading to determination of Wilting Point
October
22
23
24
25
26
27
100
90
80
70
Temperature
R. H.
Temp. F° and R. H. %
60
40
30
Days Elapsed Time 0 2 4 6 8 10 12 14 16 18 20 24 28 32 36
Date 12 14 16 18 20 22 24 26 28 30 1 3 5 7 9 11 13
October
November

Fig. 6.

Summary

(1) Fifty-eight observations of the wilting coefficient of Waipio soil using beans and sunflowers as indicator plants give a mean value of 23.4 ± 0.1.

(2) There seems to be no significant difference* between the results obtained with beans and with sunflowers, or between the results with beans by computation of the percentage of residual moisture and its actual determination by oven drying, or between successive wiltings with either species.

(3) Sugar cane leaves seemed to exhibit the unusual physiological property of being able to restore lost turgidity over night, regardless of the moisture content in the soil supporting the plant. However, a change in the transpiration rate under practically constant environmental factors was noted when the soil moisture had been depleted to the moisture content identified as the wilting coefficient with other plants. This period of the retarded transpiration was characterized by a retardation in rate of growth and by a consistent curling of the leaves.

(4) The present study of one soil only, indicates that a constant of 1.37 should be used in the denominator of the Briggs and Shantz equation for the empirical determination of the wilting coefficient, thus (for the selected Waipio soil):

$$\text{Wilting coefficient} = \frac{\text{Moisture equivalent}}{1.37}$$

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* This difference is, in all cases, less than five times the square root of the sum of the squares of the probable errors of the quantities under consideration.

The Relation of Molasses to the Micro-Biological Activity in Soil

By A. Floyd Heck

Too often the soil has been considered from the purely chemical or mineralogical standpoint as an inert mass of primary and secondary minerals, instead of the active living thing that it really is. In a recent paper, Moir (2) has recognized the biological phase of soil activity and called attention to the need for its study in Hawaiian soils. Fertile soils are not dead but are very much alive and teeming with countless millions of many kinds of organisms. Based on numbers, bacteria will take the lead in the soil population but in quantity of micro-organic material present in the soil, the fungi will usually stand first. The amount of micro-organic material in the form of yeast cells and actinomycetes will probably be intermediate, depending on the kind and amount of energy material present for their growth.

The activities of soil micro-organisms depend very much upon environmental conditions such as temperature, aeration and moisture, but of greatest importance is the amount and kind of organic material present upon which the organisms may draw for their energy supply. Proteinaceous materials are largely decomposed by bacteria, while the simpler carbohydrates, such as sugars and starches are used by both bacteria and yeasts. The destruction of the more woody tissue, consisting largely of cellulose or cellulose-like substances, is accomplished for the most part by the soil fungi and particularly certain species of the higher groups. Under any set of conditions there is always an equilibrium established between the activities of these organisms and their environmental conditions. Favorable temperature, aeration and moisture conditions cause more rapid biological activity which reduces the energy supply and a new balance is established. Under the favorable climatic conditions of Hawaii the available organic energy material has been reduced to a point where the biological activity or the "life factor" of the soil has reached a very low stage, lower, in fact, than most of the poorer soils of the mainland when placed under similar conditions.

This "life factor" or biological balance is very important in its relation to the plant nutrients and regulates to a large extent their availability to the growing plant. This is more especially true of nitrogen than either of the other elements because micro-organic material contains from 2 to 10 per cent of nitrogen. The extent of this relation with phosphorus and potassium is not known, but it is no doubt greater than many of us have thought. Although the total nitrogen may be high, in most normal soils with a normal biological balance, there is seldom more than a small amount of nitrate or ammonia nitrogen. This condition is nature's wise provision which not only protects the soil from heavy losses of nitrogen as nitrate, but also regulates the supply to the growing plant in the amounts best suited to its nutritional needs. A plant is much the same as an animal, not only requiring a balanced nutrition but also the proper amount at the proper time. In

the role of this balancing factor soil micro-organisms perform one of their greatest functions.

When molasses is placed in the soil the energy balance is disturbed and a very rapid biological action takes place because of the added energy material for the soil micro-organisms. In case of the molasses, this added energy material is sugar and other easily decomposed carbonaceous substances. The plate counts of yeasts and bacteria for a 10-ton application of molasses is given in Table I, and shows a very rapid increase in the soil population.

TABLE I

Plate Counts of Yeasts and Bacteria in Soil After the Addition of Molasses at the Rate of 10 Tons per Acre

	Millions per Gram of Soil		
	12 hours	24 hours	48 hours
Yeast—control	22.5	25.0	10.5
10 tons molasses	25.5	380.0	145.0
Bacteria—control	107.5	183.0	151.5
10 tons molasses	169.5	2020.0	3055.0

If sucrose and glucose equivalent to that in 10 tons of molasses is added to the soil a similar increase in the number of organisms takes place, except that in this case the increase is not so great. This shows that the non-saccharine carbonaceous substances also assist in the biological activity on the addition of molasses. Table II shows the increase when the sugars alone are added to the soil.

TABLE II

Plate Counts of Yeasts and Bacteria in Soil After the Addition of Sucrose and Glucose Equivalent to that in Molasses at the Rate of 10 Tons per Acre

	Millions per Gram of Soil		
	12 hours	24 hours	48 hours
Yeast—control	29.0	23.0	28.0
sugar	26.0	217.0	80.0
Bacteria—control	81.0	87.0	102.0
sugar	244.0	803.0	1620.0

These figures show a decided increase in the number of organisms between 12 and 48 hours. Although the number of bacteria present is much greater than the number of yeasts, when the size of these two organisms is considered, probably over 90 per cent of the micro-organic material produced is made up of yeast cells. These figures show the correctness of McGeorge's (1) suggestion that the rapid biological activity in the soil on the addition of molasses was due to the growth of yeasts. At the end of 48 hours about 85 per cent of the sugar applied in the molasses had been used up by the organisms, and the remaining 15 per cent was used within the next few days.

Carbon Evolution

With the destruction of the sugars and the growth of soil organisms comes a rapid rise in temperature and an evolution of carbon dioxide as a by-product. It has been found that the evolution of carbon dioxide from a soil is one of the best measures of biological activity. In the case of the molasses the bulk of the carbon dioxide is evolved during the first 48 hours, and correlates both with the number of organisms and with the temperature rise. Fig. 1 shows graphically the evolution of carbon dioxide and the temperature rise for the first 7 days from the application of molasses at the rate of 10 tons per acre.

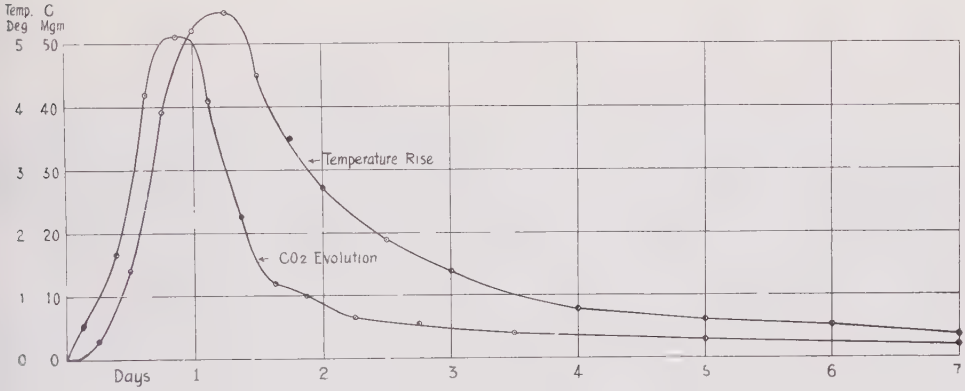

Fig. 1. Showing rise in temperature and the rate of evolution of carbon dioxide for a period of seven days after the addition of molasses at the rate of ten tons per acre 2,000,000 pounds of soil.

The carbon from the sugars is lost more rapidly than that from the non-saccharine substances. At the end of 10 days about 56 per cent of the carbon in the sugar and 35 per cent of that in the non-saccharine substances was evolved as carbon dioxide. The amount of carbon evolved as carbon dioxide during the first 10 days is governed somewhat by the amount of available nitrogen present for the organisms. With a small amount of nitrate nitrogen present 49 per cent of the total carbon in the molasses was lost during the first ten days, but if an abundance of nitrogen was present 59 per cent was evolved as carbon dioxide. This shows that nitrogen hastens somewhat the decomposition of the molasses by supplying that element to the growing organisms.

At the end of ten days all of the sugars and all traces of any pigment materials in the molasses have disappeared. If by this we may assume that the molasses has entirely decomposed and still 40 per cent of its carbon is retained in the soil, then it must have been assimilated by the soil organisms and built up into micro-organic tissue. If this is true, then at the end of ten days after the application of 10 tons of molasses to a soil there has been produced approximately 3 tons (dry basis) of living organic matter, perhaps 90 per cent of which is in the form of yeast cells. This form of organic matter is very unique and perhaps never occurs except under these peculiar conditions. In all of the writer's experience

yeasts have never been considered as a very large factor in soil fertility. When ordinary organic matter, such as green manures, are plowed down the decomposition is largely by bacteria and fungi and the organic matter left in the soil is largely inert or dead material. With the yeasts resulting from the use of molasses, the added organic material is living, high in nitrogen and very active and has the advantage over dead material that its availability will be slower and extend over a longer period of time. This is of especial value under the climatic conditions of Hawaii where decomposition goes on 12 months of the year, and dead organic material such as green manures goes out very rapidly.

The biological activity of the soil is greatly increased by the addition of molasses. Even after the sugars have all disappeared and perhaps none of the original organic substances added in the molasses are left, the soil is much more active biologically than the control soil. Between the tenth and the eighteenth day after its application from six to eight times the amount of carbon dioxide is evolved from the soil treated with 10 tons of molasses per acre than from the un-

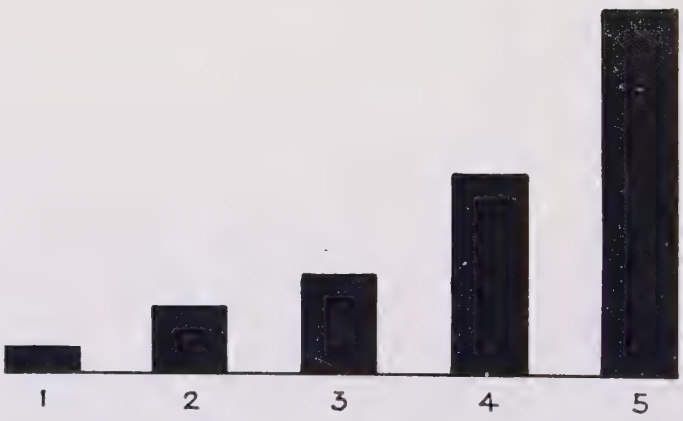

Fig. 2. The relative biological activity for the period between 14 and 18 days after a soil containing 328 p.p.m. of nitrate nitrogen is treated with varying amounts of molasses.
1. Control, no molasses.
2. 2½ tons molasses per acre 2,000,000 lbs. soil.
3. 5 tons molasses per acre 2,000,000 lbs. soil.
4. 10 tons molasses per acre 2,000,000 lbs. soil.
5. 20 tons molasses per acre 2,000,000 lbs. soil.

treated soil. This is a very good indication of the relative biological activities of the two soils. In time this spread decreases and approaches the control but perhaps reaches it only after a very long period of time, if at all. This helps to account for the long continued beneficial effects that have been observed from the use of molasses. Fig. 2 gives some idea of the relative biological activities of a soil containing 328 p.p.m. of nitrate nitrogen when treated with varying amounts of molasses.

Nitrogen Relations

Of even greater interest than the accumulation of organic matter as such, resulting from the application of molasses, or the increased biological action which

follows, is the relation which these activities bear to the nitrogen present in the soil as ammonia or nitrate. Almost immediately after the application of molasses to a soil there is a rapid disappearance of the nitrate or ammonia nitrogen present. This disappearance is almost directly proportional to the amounts of molasses used, and is equivalent to between 25 and 30 pounds of nitrogen for each ton of molasses. This nitrogen is not lost but used in the growth of the yeast cells and bacteria and built up into active living organic matter, from which it is liberated at a later period. Fig. 3 shows the disappearance of the nitrate nitrogen from a soil containing 328 p.p.m. of nitrate nitrogen, when untreated, and when treated with amounts of molasses varying from 2½ to 20 tons per acre 2,000,000 pounds of soil.

There is a very close correlation between the drop in nitrates, the carbon evolution, the rise in temperature and the increase in number of micro-organisms, so that it seems rather safe to say that the whole process is biological and that the nitrogen is changed from inorganic or nitrate nitrogen to protein or organic nitrogen during the growth of the soil organisms. This is not denitrification in the true sense of the word but only a change from the inorganic to the organic form with no resultant loss of nitrogen and has no relation to the process of nitrification.

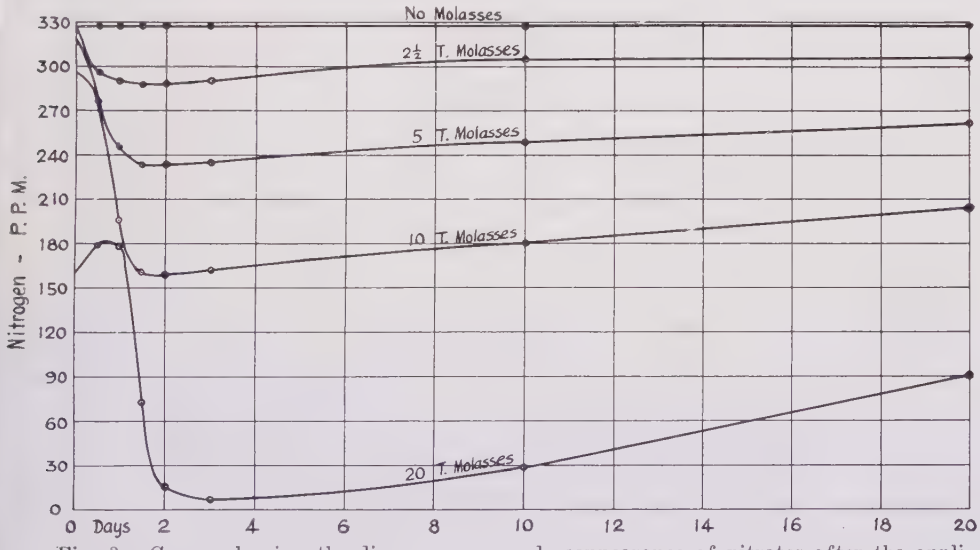

Fig. 3. Curves showing the disappearance and reappearance of nitrates after the application of molasses in varying amounts to a soil containing 328 p.p.m. of nitrate nitrogen.

For this nitrogen to become available to the growing plant it must again change to nitrate and this takes place in due course of time as the organisms die and decompose in the soil. The rate at which this takes place depends upon the amount of micro-organic material present, and this amount is governed by the amounts of molasses and nitrogen added in the beginning. If the amount of molasses is in excess of a ton for each 25 pounds of mineral nitrogen present, the nitrification rate will be slow, but if less than this amount it will be more rapid. It appears that for best results there must be something of a balance between the molasses and the mineral nitrogen. This may help to explain why the use of

molasses has sometimes given excellent results and at other times little or nothing, or delayed returns coming in the ratoon crop. There is also a physiological balance between the nitrogen as nitrate in the soil and the needs of the plant, and it appears that the combination that will furnish the nitrogen to the plant at about the rate which will best supply its assimilation needs, will give the best plant growth. In other words, a nitrification curve similar in form to the growth curve of the plant will perhaps give best results.

Conclusions

1. When molasses is applied to the soil under aerobic conditions, the sugar which it contains is decomposed and lost within three or four days and from 50 to 60 per cent of the total carbon in the molasses is lost in the first ten days.

2. There is a rapid rise in temperature corresponding to the evolution of carbon dioxide.

3. There is a rapid increase in the bacterial and yeast counts corresponding to the evolution of carbon dioxide and the rise in temperature.

4. The application of 10 tons of molasses per acre renders the soil thus treated from six to eight times as active biologically, after a period of two to three weeks, as the untreated soil.

5. Mineral nitrogen is utilized in the process of decomposition of molasses in the soil to the extent of about 25 to 30 pounds of nitrogen per ton of molasses decomposed, and a living, active micro-organic material formed in the soil which contains 4 to 6 per cent of nitrogen. Evidence indicates strongly that this organic material is largely made up of yeast cells.

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A Rapid Colorimetric Method for Phosphorus Determinations

By Ritchie R. Ward

The importance of phosphorus in studies of soil fertility, and of biological materials, has been recognized for many years. Methods for determining large amounts of phosphorus have long been known, but Taylor and Miller (1) were the first investigators to employ a colorimetric method for the estimation of small amounts.

This method depends on the blue color that is developed when the molybdate of phosphorus is reduced with phenyl hydrazine. A number of other reducing agents have been suggested (2), but stannous chloride, used by Denigès (3), and hydroquinone, used by Bell and Doisy (4) have come into most general use. The method of Bell and Doisy was subsequently modified by Briggs (5) and by Fiske and Subbarow (6), and has been used by Arrhenius in Java.

Parker and Fudge (7) studied these methods in relation to their merits for determining phosphorus in soils, and reached the conclusion that the method of Denigès is approximately five times as sensitive as that of Fiske and Subbarow.

Improvements on the Denigès method were recently made by Truog and Meyer (8), who made a detailed study of interfering substances, and worked out a procedure which eliminates such errors. They state that the improved method "appears to be one of the most satisfactory in the field of colorimetric methods and should find wide application in biology, agriculture and industry."

A large number of determinations of phosphorus are made in the Experiment Station chemical laboratory. The regular method is by precipitation as ammonium phosphomolybdate and subsequent titration, which is tedious and time consuming. A faster method of comparable accuracy was desirable; accordingly, a series of determinations was made by the colorimetric method of Truog and Meyer, and the results compared with those obtained by the regular volumetric procedure followed in this laboratory.

The reagents required for the colorimetric determination are given by Truog and Meyer as follows:

Ammonium molybdate—sulfuric acid solution: Dissolve 25 grams ammonium molybdate in 200 cc. of water heated to 60° C. and filter. Dilute 280 cc. of arsenic- and phosphorus-free concentrated sulfuric acid (about 36 N) to 800 cc. After both solutions have cooled, add the ammonium molybdate solution slowly, with shaking, to the sulfuric acid solution. After the combined solution has cooled to room temperature, dilute with water to exactly 1000 cc. This is a 10 N sulfuric acid solution containing 2.5 grams of ammonium molybdate per 1000 cc.

Stannous chloride solution: Dissolve 25 grams of $SnCl_2$. $2H_2O$ in 1000 cc. of dilute (10 per cent by volume) hydrochloric acid solution. Filter if necessary. Store in a bottle with a siphon or side opening near the bottom, arranged with a glass stopcock for delivering the solution in drops. The solution should be protected from the air by floating a layer of white mineral oil about 5 mm. thick over the surface.

Standard phosphate solution: Dissolve 0.2195 gram of recrystallized potassium dihydrogen phosphate and dilute to 1000 cc. This solution contains 50 parts per million of phosphorus and is too concentrated to use directly. A second stock solution is made by taking 50 cc. of the first stock solution and diluting to 500 cc. This second stock solution contains 5 p.p.m. and is used for making the standard solution for comparison.

A Kennicott-Sargent colorimeter was used for making the color comparisons. To make the standard solution for use in the colorimeter, take 25 cc. of stock solution containing 5 p.p.m. of phosphorus and dilute to 450 cc. with distilled water, add 20 cc. of ammonium molybdate-sulfuric acid solution and mix thoroughly by shaking in the volumetric flask. Add 30 drops of stannous chloride solution, and shake. Dilute to exactly 500 cc., shake, then wash out the colorimeter tube and plunger several times with the solution, finally filling the colorimeter tube. Insert plunger, and the colorimeter is ready for use.

Analytical procedure: In citric acid extracts precipitate the iron, aluminum, and phosphorus from solution with ammonia as usual, adding ferric chloride on which a blank has been determined, if necessary; wash thoroughly with hot water. A double precipitation is recommended. Wash the precipitate from the filter with a stream of water, taking care to include that part which sticks under the folds of the paper. Carefully dissolve with 1 to 1 sulfuric acid, using as little in excess as possible. From 1 to 5 cc. of 1 to 1 acid will be required, depending on the amount of precipitate. If the solution is more than faintly acid, bring the solution near neutrality with dilute caustic, stopping before the red color of phenolphthalein appears.

Wash the solution into a 500 cc. volumetric flask, make up to the mark, and pipette 5 cc. into a 250 cc. beaker or Erlenmeyer flask. Dilute to about 50 cc., add a piece of mossy cadmium to reduce ferric iron, and let stand several hours, or preferably over night.

Wash into a 100 cc. volumetric flask, add 4 cc. of ammonium molybdate-sulfuric acid solution, shake, add 6 drops of stannous chloride solution, make up to the mark and mix thoroughly.

The blue color appears immediately; if the color is less intense than that of the standard, pour the entire 100 cc. into the colorimeter comparison tube, and adjust the standard to match. If the solution contains more phosphorus than the standard, pour a correspondingly smaller amount into the graduated comparison tube and multiply the result by the proper factor.

If the solution contains more than twice the amount of phosphorus as the standard, it will be necessary to take an aliquot and repeat the procedure. Maintain the same amount of molybdate (4 cc.) and stannous chloride (6 drops) in 100 cc. final solution. It is not advisable to use a stronger standard, as the color developed will be too intense to allow accurate matching.

The volume of standard required to match the solution, multiplied by 0.0001145, gives the per cent citric acid soluble phosphoric acid, when the total iron-aluminum-phosphorus precipitate is made up to 500 cc. and a 5 cc. aliquot is taken.

Comparison with the standard should be made within 10 minutes after adding the stannous chloride. It has been found convenient to make up a separate standard

for each set of ten determinations; it is possible to make the ten comparisons within the allotted time.

Truog and Meyer outline the following general precautions:

> Reagents, filter paper, water, and glassware often contain appreciable amounts of phosphorus and arsenic. Blank tests should be made frequently in which all the reagents and glassware come into play; there should not be produced more than a very faint blue color, if everything is satisfactory. New glassware should be weathered for 24 hours in dichromate—sulfuric acid mixture. It is absolutely essential that each lot of new reagents be tested.

Munktell's No. 0 filter paper has been found to be practically free from phosphorus and arsenic.

Experimental results: A test was first made to determine whether the results obtained by the colorimetric method were reproducible. A series of duplicate determinations was run on a set of 35 soils; the results are presented in Table I. It will be seen from these figures that uniformly good checks can be obtained.

A comparison was next made between the results obtained by the colorimetric method and those obtained by the regular volumetric procedure. These figures are shown in Table II. In column A are shown the results using the regular method; in column B are shown the colorimetric results on the corresponding soil.

The straight line which best represents these points on a graph was calculated by the method of least squares. It is a line of slope 0.827, and if we let

$x =$ per cent P_2O_5 as determined volumetrically,
$y =$ per cent P_2O_5 as determined colorimetrically,

the equation of the curve is

$$x = \frac{y - 0.0004}{.827}$$

In column C the value of y as given by the equation is presented, and the difference of this value from that actually observed appears in column D. In column E the corresponding percentage differences are shown.

TABLE I

Per Cent P_2O_5, Run 1	Per Cent P_2O_5, Run 2	Per Cent P_2O_5, Run 1	Per Cent P_2O_5, Run 2
.016	.016	.032	.032
.028	.027	.021	.021
.038	.037	.0096	.0092
.045	.046	.0073	.0082
.071	.079	.012	.011
.030	.029	.019	.017
.052	.056	.040	.039
.0087	.0082	.017	.017
.027	.026	.0069	.0064
.022	.022	.0055	.0050
.0082	.0092	.0046	.0050
.025	.025	.013	.013
.011	.011	.0073	.0082
.0055	.0060	.0087	.0092
.0078	.0079	.0078	.0078
.043	.043	.0041	.0041
.012	.012	.0055	.0060
.027	.026		

These differences are then treated by the statistical method. It is found that the standard deviation of a single percentage difference is

$$\text{S. D.} = \sqrt{\frac{1233}{25—1}} = 7.2$$

and the corresponding probable error

$$\text{P. E.} = 0.6745 \times 7.2 = 4.8$$

This means that the probable error of calculating the per cent phosphoric acid from the equation is 4.8 per cent. To illustrate, if a colorimetric determination gave a value of 0.0035 per cent P_2O_5, the volumetric determination should give a value of 0.0038 ± 0.0002.

It is believed that this error is less than the respective errors of sampling the soil, extracting with citric acid, and finally, interpreting the results in terms of fertilizer practice.

TABLE II

A	B	C	D	E	F	G
Per Cent P_2O_5 Volumetrically	Per Cent P_2O_5 Colorimetrically	Per Cent P_2O_5 Calc. From Equation	Difference	Per Cent Difference		
Obs. x	Obs. y	Calc. y	Obs. y—Calc. y	Obs. y—Calc. y / Calc. y	Difference of E from Average E	F2
.0014	.0019	.0016	.0003	18.2	8.2	67.2
.0013	.0017	.0015	.0002	13.3	3.3	10.9
.0013	.0016	.0015	.0001	6.7	3.3	10.9
.0016	.0016	.0017	.0001	5.9	4.1	16.8
.0023	.0023	.0023	0	0	10.0	100.0
.0024	.0021	.0024	.0003	12.5	2.5	6.3
.0026	.0022	.0026	.0004	15.4	5.4	29.2
.0027	.0023	.0026	.0003	11.5	1.5	2.3
.0042	.0037	.0039	.0002	5.1	4.9	24.0
.0045	.0044	.0041	.0003	7.3	2.7	7.3
.0056	.0038	.0050	.0012	24.0	14.0	196.0
.0077	.0066	.0059	.0007	11.9	1.9	3.6
.0091	.0072	.0079	.0007	8.9	1.1	1.2
.0098	.0069	.0085	.0016	18.8	8.8	77.4
.0100	.0086	.0087	.0001	1.1	8.9	79.2
.0110	.0096	.0095	.0001	1.1	8.9	79.2
.0115	.0110	.0099	.0011	11.1	1.1	1.2
.0117	.0095	.0101	.0006	5.9	4.1	16.8
.0120	.0087	.0103	.0016	15.5	5.5	30.3
.0124	.0133	.0107	.0026	24.3	14.3	204.5
.0140	.0120	.0120	0	0	10.0	100.0
.0166	.0164	.0141	.0023	16.3	6.3	39.7
.0314	.0292	.0264	.0028	10.6	0.6	.4
.0478	.0395	.0399	.0004	1.0	9.0	81.0
.0574	.0464	.0479	.0015	3.1	6.9	47.6
				$\Sigma = 249.5$		$\Sigma = 1233.0$
				Avg. $= 10.0$		

A test was next made to determine the ability of different observers to match color intensity, as required in this method. Four different persons matched the solution and standard on the same sample. The volume of standard necessary to match the sample was found to be 76 cc. in one case, and 84, 87 and 92 cc. in the others. These readings differ widely, yet each observer was able to check his own reading within 1 or 2 per cent.

In view of the fact that the personal equation enters so prominently into the matter of matching color intensity, it will be necessary for each analyst to determine his own curve, and advisable that he also determine the probable error of a single observation.

The above results appear to indicate that the colorimetric method may be used in determining phosphorus in soil extracts, without introducing errors larger than others already present.

The method is rapid and convenient, and is particularly useful when small amounts of phosphorus are to be determined.

Summary

(1) A rapid colorimetric method for determining phosphorus has been described.

(2) The results obtained have been shown to be reproducible.

(3) An equation for calculating the per cent of phosphorus as indicated by the standard method, from the results of the colorimetric method, has been developed.

(4) The influence of the personal equation on the method has been noted, and means for obviating the error so introduced have been presented.

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Boric Acid Absorption of Kjeldahl Nitrogen

By Arthur S. Ayres

The methods employed at this Station for the determination of nitrogen in fertilizers involve its reduction, from various forms, to ammonia. The ammonia, thus formed, is subsequently distilled through cooling coils into an accurately measured quantity of standard sulfuric acid solution. Back-titration with standard sodium hydroxide solution gives the amount of acid required to neutralize the ammonia distilled from the sample.

In 1913, Winkler (1) proposed the substitution of boric acid for sulfuric in the fixation of ammonia distilled over in the course of the Kjeldahl method for the determination of total nitrogen. By this substitution the necessity for back-titration is eliminated and the ammonia in the fixing solution is titrated directly with a standard acid. Scales and Harrison (2) investigated this method and found it to be equally as accurate as the standard sulphuric acid absorption. Spears (3) in 1921 conducted a series of sixty determinations on feed stuffs by both methods. The agreement obtained was very satisfactory.

The several advantages of boric acid over sulfuric acid in nitrogen determinations, as found by these investigators, has led us to consider its adoption in fertilizer analysis. A series of comparative tests of the two methods has been made and it is the purpose of this paper to present and briefly discuss the results.

In order to obtain a fair comparison of the two methods laboratory samples of fertilizers, taken largely in the order received, were analyzed as usual and checked in duplicate against the newer procedure. These samples contained nitrogen in widely varying percentages in one or more of the following forms: ammonia nitrogen, nitrate nitrogen and organic nitrogen. Routine separations, reductions, digestions, etc., were all made by official (A. O. A. C.) methods. Distillations were carried out in accordance with the regular laboratory procedure. In the case of the samples run by the sulfuric acid method the distillate was received into a sufficient excess of 0.2 N sulfuric acid to effect complete neutralization, using methyl red indicator. Where boric acid was used the distillate was received, at room temperature, into 50 cc. 4 per cent boric acid solution. Spears (3) found that 0.095 gram of nitrogen, as ammonia, could be recovered with accuracy from the distillate when this quantity of boric acid was used. This result was obtained without the use of glycerin which Adler (4) recommends. The ammonia was led as deeply as possible into the fixing solution during the first fifteen minutes of distillation, after which the condenser tube was raised so that the subsequent distillate would serve to rinse the tip. Blanks were run using all reagents. The standard acid employed as a neutralizing agent in the sulfuric acid method and for the direct titration in the boric acid method, was 0.2 N sulfuric acid.

Various indicators were tested in conjunction with the new reagent. Congo red was found to be the most satisfactory, although choice of indicator is somewhat

a matter of personal taste. Spears (3) recommends the use of bromophenol blue in this titration, using artificial light.

The indicator employed in this laboratory for the determination of nitrogen by the sulfuric acid method, is methyl red. In the determination of ammonia nitrogen, Congo red has a decided advantage over this indicator. The base used in the ammonia-nitrogen-determination is magnesium oxide, which contains magnesium carbonate as an impurity. The carbon dioxide carried over during the distillation interferes with the proper action of methyl red, as an indicator, and hence mus be removed prior to titration. The use of Congo red renders this step unnecessary Where boric acid is used as the fixing agent no attempt should be made to heat the distillate before titrating.

The results obtained by the two methods (see Table) show very close agreement in practically all cases. Other comparative tests by Van Brocklin, Shepardson and the writer, show equally close agreement. The average of all values shown in the table indicates that there is no systematic error involved. The averages of the deviations from the mean, 0.013 for the sulfuric acid method and 0.014 for the boric acid method, show no significant difference. The probable error, calculated from the data, is 0.0092 for the former method and 0.0100 for the latter, which is identical for practical purposes. The results appear to be equally satisfactory for each of the three forms in which nitrogen was determined, namely, ammonia nitrogen, nitrate nitrogen and organic nitrogen.

TABLE OF RESULTS

Sample	Per Cent Nitrogen by H_2SO_4 Absorption	Average	Per Cent Nitrogen by H_3BO_3 Absorption	Average
1	7.06		7.01	
	7.00	7.030	7.07	7.040
2	10.26		10.20	
	10.22		10.17	
	10.26	10.247	....	10.185
3	2.91		2.91	
	2.89		2.90	
	2.90	2.900	....	2.905
4	2.27		2.29	
	2.24	2.255	2.26	2.275
5	11.66		11.62	
	11.54		11.57	
	11.64	11.613	....	11.595
6	8.66		8.66	
	8.66	8.660	8.66	8.660
7	4.34		4.28	
	4.30	4.320	4.24	4.260
8	5.46		5.56	
	5.50	5.480	5.46	5.510

9	0.74		0.71	
	0.74		0.70	
	....	0.740	0.69	0.700
10	0.51		0.49	
	0.50		0.49	
	....	0.505	0.50	0.493
11	0.53		0.56	
	0.54	0.535	0.54	0.550
12	0.76		0.74	
	0.76	0.760	0.74	0.740
13	0.53		0.51	
	0.52	0.525	0.51	0.510
Average		4.27		4.26

The chief advantages of the new method over the old one are:

(1) Employment of one standard solution instead of two. This saves time and eliminates one possible source of error.

(2) But one accurate measurement is required instead of two. Time is again saved and the possibility of slight errors in reading the burette eliminated. Since the absolute amount of boric acid in the receiving flask need not be accurately measured, an unskilled assistant may do this part of the work, where a large number of nitrogen determinations are to be made. Scales and Harrison (2) found that this rough measurement of the acid saves from one-fourth to one-third of the time required to obtain equally accurate results by the old methods.

(3) The strength of the standard acid, and weights of samples taken can be so adjusted that the percentage of nitrogen can be read directly from the burette, or else obtained by the simplest mental arithmetic.

From a consideration of the advantages of the new method over the old and from the foregoing analysis of data, it is felt that the substitution of boric acid for sulfuric acid, as a fixing agent in the determination of nitrogen in fertilizers, is well justified.

Summary

1. The methods of nitrogen determination, as heretofore employed at this Station, involve the distillation of ammonia into an accurately measured quantity of standard sulfuric acid. The excess acid is then back-titrated with standard sodium hydroxide solution and the percentage of nitrogen in the sample calculated from the difference thus obtained.

2. The boric acid method of nitrogen determination, in which boric acid solution is substituted for sulfuric acid as the fixing agent, permits the direct titration of the ammonia in the distillate with standard acid.

3. Comparative tests on nitrogen in fertilizers have been determined by both methods in this laboratory. The agreement between the two sets of results thus obtained is very satisfactory.

4. It was found that the boric acid absorption is a time-saving expedient which may be employed without any sacrifice of accuracy.

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Forestry in Formosa

By J. P. Martin

While en route to Java to attend the Third Congress of the International Society of Sugar Cane Technologists, 1929, the delegates from Hawaii had the opportunity, through the courtesy of the government of Formosa, to observe some of the various Formosan industries.

Among the outstanding industries of Formosa is lumbering, and the forests are under the protection of the government. A trip was made to the forest of Arisan, which is one of the three principal forests. The illustrations accompanying this article were taken while visiting the Arisan forest. In the higher elevations strange people, known as head-hunters, still inhabit the primeval forests. The savage proclivities of these primitive people are now kept under control and only an occasional uprising occurs.

The government of Formosa published, in 1926, a book entitled: *Progressive Formosa*. The following paragraphs, dealing with forestry in Formosa, are taken *verbatim* from this book:

Well-nigh one-half of the surface of the island being covered with high mountains rising to a height of 6-9,000 ft. to 12-13,000 ft. above sea-level, there exists a large area of magnificent primeval forest.

The forests bordering on the plain had, under the Chinese regime, been to a great extent denuded of trees, owing partly to reclamation for agricultural purposes, and partly to fires and indiscriminate felling for timber and fuel, without any attempt at re-afforestation. The re-afforestation work was taken in hand after the advent of the Japanese, but has not yet made any notable progress. Accordingly the unexploited primeval forests are now only to be found in the rugged high mountains far beyond the access of civilized settlers. The forests of Taiwan, owing to their geographical position, geological qualities, and climatic conditions, comprise an amazingly large variety of trees belonging to the tropical, sub-tropical, temperate and even the semi-artic zones. It is no wonder that the Portuguese navigators looking up at the verdant mountains from the high exclaimed: "Isle of Beauty!"

Among many large forests so far discovered, the most renowned are the Arisan, Hassenzan and Dakusui-kei forests, where exploitation is being actively carried on under the Government Forestry Bureau. In 1923, the volume of timber disposed of by the Government amounted to over 298,990 koku,* representing 3,559,000 yen† in value. Of the three great forests, the Arisan forest is the most important, standing foremost in respect of extent, wealth and beauty, and is fast becoming known to Japanese and Westerners as well. Arisan is the name given to a range of mountains stretching westward from Mt. Niitaka, and is situated about forty miles east of Kagi. The forest lies at an altitude ranging from 2,800 ft. to 8,700 ft. above sea-level, covering an area of 11,000 chobu (27,000 acres). The timber obtainable from the forest is estimated at 22,000,000 board feet. As for the distribution of the predominant trees, benihi (*Chamaecyparis formosensis*) grows mingled with other trees at an altitude of 6,000 feet above sea level, and from 7,000 feet upwards are found in turn, woods all composed of benihi and hinoki (*Chamaecyparis obtusa*), while tsuge (*tsuge formosa*) and himekomatsu (*pinus parviflora*) are found min-

* Koku = 9.8274 cubic feet.

† Yen = 50¢.

gled together at 8,000 ft. These innumerable trees form the magnificent primeval forests that have remained sacred and untouched from time immemorial.

The exploitation of the Arisan forests was started as a private undertaking by Fujita and Co., but was transferred to the Government through purchase in 1910. A railway more than 54 miles in length has been constructed for the transport of timber from the forests. The railway section from Kagi to Takezaki is opened to general traffic, but the line beyond Takezaki is very steep and is not open for public use.

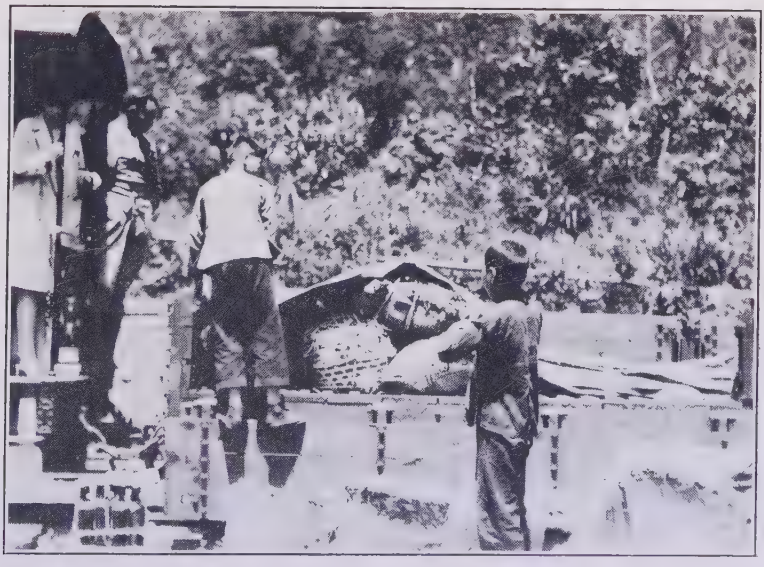
Special permission will, however, be given to visitors to Mt. Arisan to avail themselves of the line. The forest railway steams up, crawling along rugged mountains and sharp cliffs in a spiral fashion, and reaches the high elevation of 8,000 feet. The train in its journey passes through more than 73 tunnels as well as innumerable cuttings. The grand kaleidoscopic views and exciting experiences one can enjoy while ascending lofty mountains and traversing deep valleys are simply wonderful and quite beyond description.

By means of aerial cables, the timber felled is conveyed and gathered at points accessible to railway stations, and then transported down to the saw mills and Kagi, a distance of forty miles. The volume of timber obtained during 1923 amounted to 190,332 koku corresponding to the value of 2,447,592 yen.

The lumbering business figures show that the Arisan forests alone are capable of yielding more than half of the whole production of the island and this vouches for a further development of the lumber industry in the same forests.

These primeval forests contain large numbers of old trees which have passed beyond their mature stage. The Government, therefore, has formed the plan of renovating the Arisan forests by felling aged trees and re-afforesting them afterwards. The Government is also carrying out afforestation schemes in various districts, and is giving encouragement to private parties to engage in the same useful work. During 1923, the extent of newly planted land afforested both by the government and by private parties amounted to approximately 121,777 chobu.

En route to the summit of Mt. Arisan.



Freight being transported to the lumber camps.

Reforestation of slopes of Mt. Arisan as viewed from the train.

Village built entirely of bamboo, on the way to Mt. Arisan.

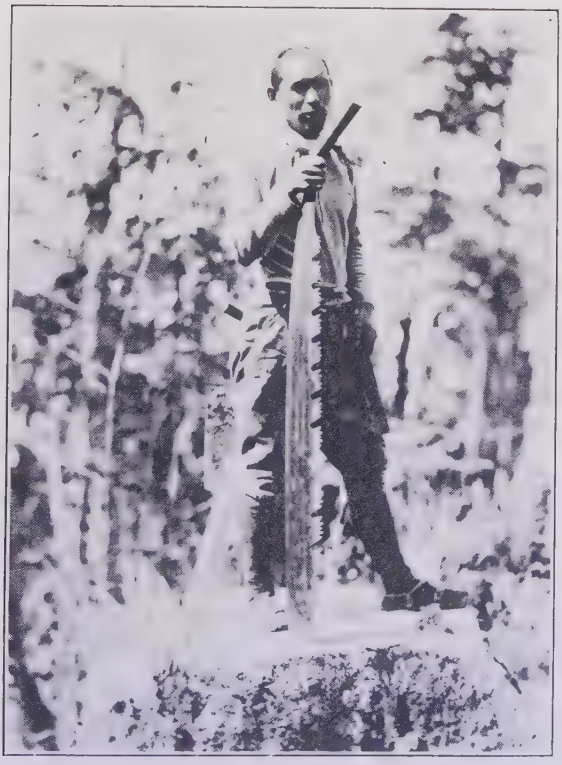
Rugged peaks.

Side-hill forests, Mt. Arisan.

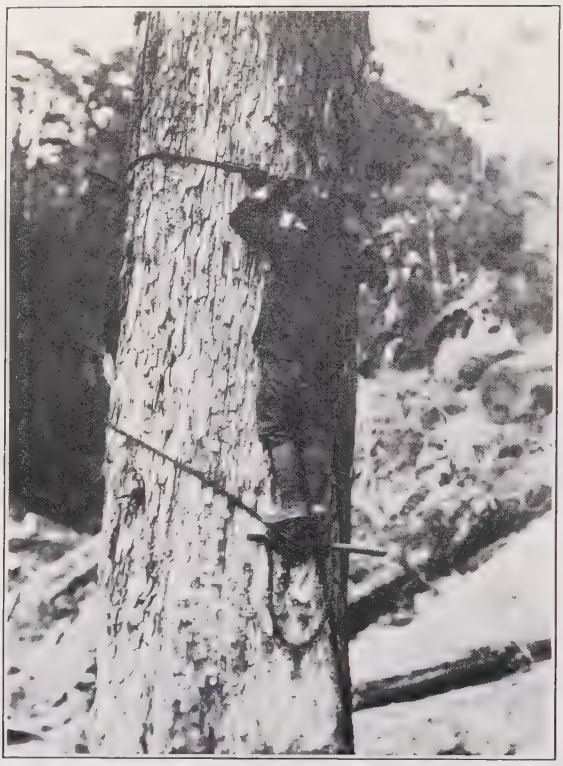
Mountain scenery.

Heavy forests; elevation 8000 feet.

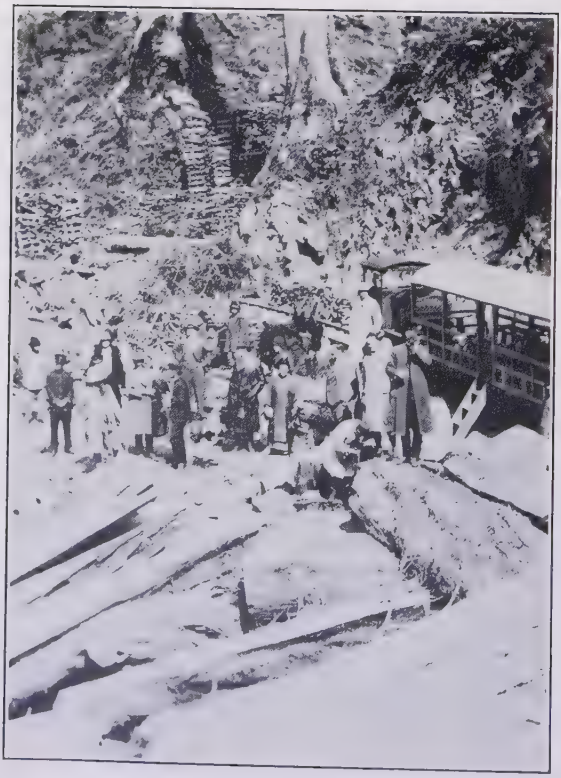
Newly planted forests; elevation 8000 feet.



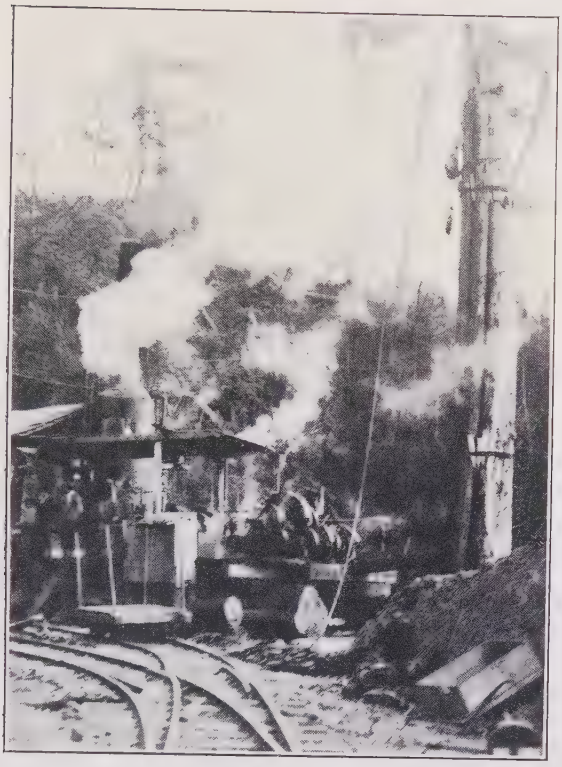
A lumberman who had just felled a large tree.



A tree climber.



Some of the delegates at a logging camp, Mt. Arisan.



A donkey engine for snaking logs to the railroad station.

A large log being transported from the forest to the railroad station by aerial cables.

Railroad passing through a forest that has been logged.

Peaceful head-hunters.

"Close-up" of the women head-hunters.

"Close-up" of the men head-hunters.

The Decomposition of Carbohydrates in Water-Logged Soils, and the By-Products Remaining in the Soil

The investigations conducted by V. Subrahmanyan are interesting to those planters who are retaining considerable amounts of cane trash in the fields and to all who are using, or are considering the use of, molasses as a soil amendment. The work, done at Rothamsted Experimental Station and reported in *The Journal of Agricultural Science,* Vol. XIX, October, 1929, is herein briefly reviewed.

Potassium nitrate was added in varying amounts to 100-gram samples of soil, free of all organic matter, and incubated for several days. On leaching these soil samples with water practically all of the applied nitrate was recovered in the leachings, which indicates that there could be no denitrification or utilization of nitrogen by soil organisms in the absence of sugar or other carbohydrates.

When potassium nitrate was added in the same manner, but together with varying amounts of glucose, the nitrates usually disappeared by the third day of incubation. The nitrates in the soil had been changed by the activity of various soil organisms into organic forms of nitrogen and had become part of the living protoplasm of the bodies of these soil organisms. After the available energy materials in the form of sugars or other carbohydrates in the soil have become exhausted, a gradual decomposition of the resulting dead bodies ensues, followed by nitrification processes which convert the protein or organic nitrogen contents of the organisms into nitrates again. In the form of nitrates, this nitrogen may be readily absorbed by growing plants.

The utilization by soil organisms of the applied nitrates and sugars was accompanied by a rapid decrease of the free oxygen dissolved in the overlying water. The rate of utilization of oxygen increased in proportion to the concentration of sugars in the water and soil. This indicated that the soil organisms utilized considerable quantities of oxygen in their growth. As the oxygen content of the water decreased there was an observed parallel increase in carbon dioxide content of the solution which indicated that both carbon and oxygen were being utilized by the soil population. In this instance the carbon was derived from the sugar dissolved in the soil solution.

There was an observed increase in acidity of the soil solution and surface water resulting directly from the activities of the soil organisms. This reaction would be expected, in part at least, as the result of the concentration of carbon dioxide dissolved in the water forming carbonic acid.

Another factor which accounted for the increased soil acidity was the formation in the soil of large quantities of lactic acid as the result of the activities of soil bacteria. The author states that 30 or 40 per cent of the sugar decomposed was converted into lactic acid. Later on in the process of incubating these soils the amounts of lactic acid decreased and the amounts of acetic acid increased.

The acetic acid was also a direct product of the breaking down of sugars by soil organisms. In this instance the acetic acid had been formed through oxidation of the lactic acid.

Soil samples to which had been added, not glucose, but solely lactic acid, were water-logged and incubated. Soil micro-organisms converted this lactic acid to acetic acid.

In soil samples to which sugar had been added and the samples incubated, not only lactic and acetic acids were determined quantitatively, but also butyric acid was determined as present in relatively large quantities.

Thus the author shows that the sugar added as glucose to the soils incubated in a flooded condition, was eventually converted to lactic, acetic and butyric acids and about 20 to 25 per cent eventually became carbon dioxide. These organic acids, formed by microbial activities in these soil solutions, eventually were converted to lower forms of carbon compounds by certain soil organisms, presumably reaching a final form as carbon dioxide.

Under Hawaiian conditions where water-logging or continued flooding does not normally occur, the decomposition of cane trash and of molasses probably proceeds much more rapidly. The progressive steps in the process of decomposition are doubtless the same while the end result is reached probably much sooner under our conditions of better aeration.

C. C. B.

The Pre-Harvest Sampling of Cane by Refractometer at Ewa Plantation Company

By J. D. Bond

In the hope of materially increasing the accuracy and reliability of our pre-harvest cane samples, particularly in respect to the number of samples to be obtained, studies on the use of the refractometer in sampling cane were instituted in September, 1927.

Technique

An Abbe refractometer equipped wi'h a scale to read directly in per cent solids as applied to sugar solutions, was employed in obtaining the data reported here. The instrument was adjusted to read at 28° C. and was maintained at this temperature, within limits of not over half a degree, by using the water jacket. The adjustment was checked frequently by the use of sugar solutions of known concentrations.

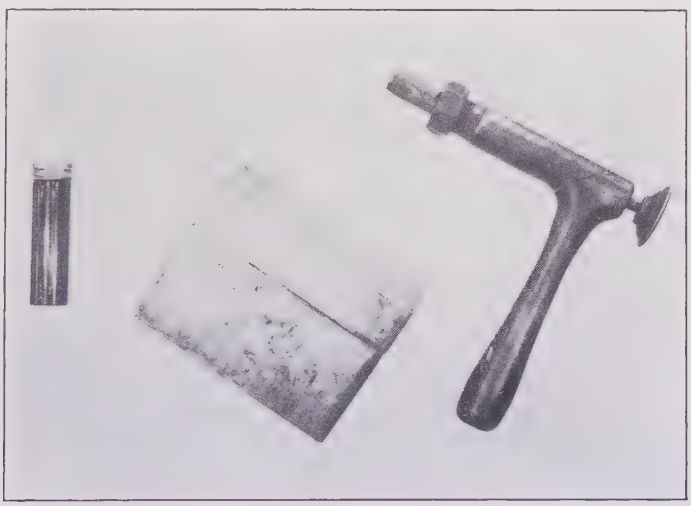


Fig. 1. (From left to right.) Vial used to receive individual borings; tin box to receive borings in lots of a hundred or more; and the sampling "gun" used in this work.

At the start of this work, ordinary cork borers were used to obtain the samples. This was unsatisfactory, however, and our machine shop subsequently developed a small sampling "gun" (Fig. 1) with replaceable cutting tips. The sample plug, about 5/16 inch in diameter and ¾ inch in length, was ejected by a small plunger. Where the solids were determined on the samples individually, each plug was dropped into a small homeopathic vial. Canvas "cartridge" belts were used to receive these vials in field work (Fig. 2). In later work, when a hundred or more

borings constituted a sample, these were collected in tin boxes, 3½ inches long, 1½ inches wide and 2½ inches deep. A close-fitting cover was designed to cut down moisture losses due to evaporation, and when necessary to keep the samples over night the box and cover were taped securely together before being placed on ice.

In the laboratory, the juice from single borings was expressed by a broad-jawed pair of pliers into the same vial, mixed, and a few drops placed on the lower prism of the refractometer. Readings were recorded directly by a small adding machine operated by the analyst. When a hundred or more borings constituted a sample, the laboratory screw press was utilized. Enough juice was expressed in this way for the determination of the polarization.

Fig. 2. Showing the use of the sampling "gun" and the belts used to receive vials.

Point of Sampling on the Stalk

In order to determine that portion of the stalk most suitable for sampling, according to the method outlined above, preliminary work was concerned with the variation of the juice solids from internode to internode throughout the stalk length. The work was done on stalks brought in as regular pre-harvest samples to be crushed in the small sample mill. The fields sampled were from 15 to 18 months old at sampling and were approaching, or at, maturity. A total of 153 stalks were used in this preliminary study. In order to sample only that portion of the stalk coming under the category of millable cane, the first boring for the refractometer was taken at the eighth internode, counting that immediately below the node to which the leaf with the topmost visible dewlap was attached, as the first. In practice, this point was determined by counting down eight leaves and

thus determining the first internode to be sampled. One boring was taken approximately at the center of each internode, dropped into appropriately labelled vials and sent to the laboratory.

The great variation in the number of internodes per stalk, necessitated a grouping of the data by stalks according to the number of internodes. The data have been summarized in Table I, each group including a range of five internodes in the total number noted. Within each of the groups of Table I, variations are found, further, dependent upon the relative maturity of the stalks as evidenced by the concentration of total solids in the juice. Assuming that the arithmetical average of all the determinations per stalk will be roughly proportional to the total expressed juice of that stalk, Tables II and III have been arranged from the data of Table I. In Table II, average solids from 16.00 to 17.99 per cent have been considered; and in Table III from 18.00 to 19.99 per cent, the same grouping of Table I being otherwise followed.

TABLE I

Variation of Stalk Solids by Internodes--Averages According to the Number of Internodes per Stalk

Internodes	27-31	32-36	37-41	42-46	47-51	52-56	57-62
No. Stalks Averaged	5	26	37	31	32	16	6
8	15.48	12.87	13.05	12.09	11.90	12.12	11.33
9	16.14	14.53	14.18	13.37	13.38	13.54	13.08
10	16.98	15.32	15.14	14.34	14.19	14.38	14.20
11	17.12	15.80	15.86	15.11	14.77	14.89	15.02
12	17.12	16.35	16.20	15.64	15.20	15.34	16.20
13	17.90	16.80	16.59	15.93	15.73	15.61	16.72
14	17.96	17.15	16.92	16.31	16.08	16.01	17.03
15	18.18	17.35	17.10	16.46	16.30	16.22	17.05
16	18.52	17.42	17.28	16.64	16.56	16.14	17.15
17	18.46	17.82	17.57	16.70	16.69	16.18	17.40
18	18.58	18.09	17.37	16.77	16.61	16.38	17.65
19	18.32	18.15	17.56	16.82	16.68	16.39	17.87
20	18.46	18.13	17.67	17.04	16.79	16.47	18.02
21	18.62	18.13	17.67	16.86	16.88	16.52	18.08
22	18.70	18.17	17.70	16.98	16.96	16.51	17.85
23	18.90	18.35	17.73	16.93	16.98	16.53	17.72
24	19.06	18.43	17.78	17.02	17.06	16.69	17.78
25	19.10	18.47	17.86	16.99	17.12	16.75	17.68
26	19.40	18.64	18.03	17.04	17.15	16.85	17.72
27	19.34	18.88	18.21	17.26	17.36	17.00	17.88
28	19.64	19.00	18.22	17.35	17.49	16.99	17.77
29		19.15	18.26	17.45	17.61	17.08	17.88
30		19.39	18.39	17.66	17.66	16.99	18.08
31		19.41	18.55	17.65	17.76	17.16	18.05
32		19.51	18.58	17.75	17.81	17.36	17.98
33			18.75	17.74	17.80	17.39	18.27
34			18.86	17.95	17.88	17.29	18.28
35			19.05	17.98	17.93	17.26	18.30
36			19.13	18.11	18.00	17.42	18.57
37			19.16	18.22	18.03	17.48	18.43

Internodes	42-46	47-51	52-56	57-56
38	18.18	18.30	17.69	18.62
39	18.20	18.32	17.94	18.58
40	18.32	18.31	18.06	18.08
41	18.33	18.31	18.03	18.50
42	18.34	18.35	17.96	18.52
43		18.47	18.13	18.45
44		18.44	18.04	18.55
45		18.44	18.19	18.33
46		18.41	18.04	18.47
47		18.45	18.09	18.28
48			18.24	18.13
49			18.26	18.62
50			18.21	18.22
51			18.21	18.43
52			18.21	18.55
53				18.63
54				18.63
55				18.77
56				18.77
57				18.98

5,533 Total Solids Determinations from 153 stalks.

TABLE II

Variation of Stalk Solids by Internodes—Averages According to the Number of Internodes per Stalk—Averages of All Determinations per Stalk Ranging from 16.00 to 17.99 Per Cent Solids

Internodes	32–36	37–41	42–46	47–51
No. of Stalks Averaged	11	13	9	11
Average All Stalks	17.06	16.87	16.79	16.90
8	11.96	12.38	11.43	11.65
9	13.51	13.82	13.04	12.89
10	14.57	15.10	14.43	13.73
11	15.01	15.48	15.12	14.43
12	15.57	15.70	15.81	14.65
13	15.94	15.98	16.00	15.23
14	16.41	16.18	16.42	15.56
15	16.46	16.41	16.53	15.65
16	16.52	16.59	16.69	15.95
17	17.05	16.93	16.72	16.19
18	17.37	16.82	16.86	15.99
19	17.80	17.16	17.06	16.15
20	17.78	17.02	17.18	16.32
21	17.85	16.86	16.68	16.35
22	17.92	16.88	16.90	16.53
23	18.03	16.90	16.87	16.68
24	18.00	17.02	16.83	16.91
25	18.08	17.21	16.84	16.85
26	18.25	17.31	16.60	17.01
27	18.49	17.45	16.83	17.25
28	18.56	17.58	16.84	17.37
29	18.58	17.57	17.06	17.44
30	18.78	17.71	17.34	17.53
31	18.95	17.84	17.34	17.74
32	19.15	17.88	17.54	17.94
33		18.16	17.57	17.81
34		18.25	17.76	17.97
35		18.47	17.74	18.01
36		18.68	17.94	18.08
37		18.85	18.12	18.10
38			17.99	18.47
39			18.12	18.59
40			18.26	18.41
41			18.46	18.42
42			18.66	18.65
43				18.69
44				18.60
45				18.81
46				18.70
47				18.69

TABLE III

Variation of Stalk Solids by Internodes—Averages According to the Number of Internodes per Stalk—Averages of All Determinations per Stalk Ranging from 18.00 to 19.99 Per Cent Solids

Internodes	32–36	37–41	42–46	47–51
No. of Stalks Averaged	12	13	11	5
Average All Stalks	18.85	18.81	18.68	18.61
8	13.41	14.50	13.85	11.96
9	15.53	15.34	15.08	13.94
10	16.56	15.90	16.05	14.50
11	17.13	17.20	16.85	15.44
12	17.61	17.53	17.15	16.08
13	18.28	18.15	17.68	17.02
14	18.53	18.57	18.07	17.44
15	18.83	18.73	18.15	17.70
16	18.82	18.86	18.36	18.20
17	19.15	19.26	18.45	18.40
18	19.51	18.68	18.33	18.34
19	19.33	18.78	18.30	18.44
20	19.31	19.06	18.46	18.50
21	19.30	19.11	18.53	19.02
22	19.33	19.22	18.61	19.14
23	19.53	19.30	18.65	19.14
24	19.54	19.25	18.81	19.04
25	19.73	19.23	18.76	19.20
26	19.79	19.47	18.89	19.12
27	20.01	19.68	19.20	19.56
28	20.16	19.57	19.45	19.54
29	20.37	19.48	19.50	19.82
30	20.57	19.69	19.65	19.56
31	20.42	19.81	19.58	19.66
32	20.43	19.77	19.62	19.66
33		19.88	19.65	19.64
34		19.95	19.93	19.68
35		20.12	19.94	19.64
36		20.13	19.97	19.62
37		20.09	20.02	19.72
38			20.00	19.84
39			19.94	19.90
40			19.91	20.14
41			19.70	20.00
42			19.66	19.88
43				19.76
44				19.76
45				19.58
46				19.44
47				19.30

There is evidently a tendency, though not entirely consistent, for the longer stalks to have lower solids in the juice at the corresponding internode, counting from the top down; or, in other words, the shorter stalks have the higher solids, at the corresponding internode from the top than the longer stalks, both yielding approximately the same normal juice. In the longer and more mature stalks, the

solids reach a maximum a few internodes above the base after which the solids decrease toward the base. In the less mature stalks the solids increase progressively toward the base.

In order to gain a general idea of the variation of solids from internode to internode, all the data of Table I have been averaged, by internodes for 15 internodes from the top down and again from the base up. These averages are given in Table IV.

TABLE IV

Internode Averages—Per Cent Solids in Juice

Internode Number			
Top to Bottom	Bottom to Top	From Top Toward Bottom	From Bottom Toward Top
8	1	12.50	18.81
9	2	13.86	18.76
10	3	14.75	18.74
11	4	15.38	18.62
12	5	15.84	18.57
13	6	16.26	18.47
14	7	16.60	18.37
15	8	16.79	18.28
16	9	16.94	18.24
17	10	17.13	18.15
18	11	17.16	18.06
19	12	17.24	18.00
20	13	17.35	17.94
21	14	17.35	17.86
22	15	17.39	17.76

The rapid increase in solids from the top down, until about the twentieth internode (Fig. 3), is evident. The decrease in solids from the base toward the top appears gradual, though, as previously noted, in the longer, more mature stalks, a maximum point is reached in this range, followed by a decrease.

In selecting a point of sampling, it is essential to designate a portion of the stalk throughout which the rate of change of solids by internodes is not excessive. Obviously, we cannot consider that portion of the stalk immediately below the top, until about the twentieth joint. It is not, furthermore, practicable to go much beyond this point under our conditions since the tracing of stalks imbedded in trash and overlain with other stalks is difficult. On the other hand, if we choose to use the base of the stalk as a point of reference, the number of internodes to be counted must be as small as possible for the same reason and yet great enough to overstep the point of maximum solids found in the longer, more mature stalks. Arbitrarily, we have chosen the tenth internode from the base and the twentieth from the top for the comparisons of the following table, the averages being obtained without respect to the stalk length. Stalks of less than 32 internodes have been excluded.

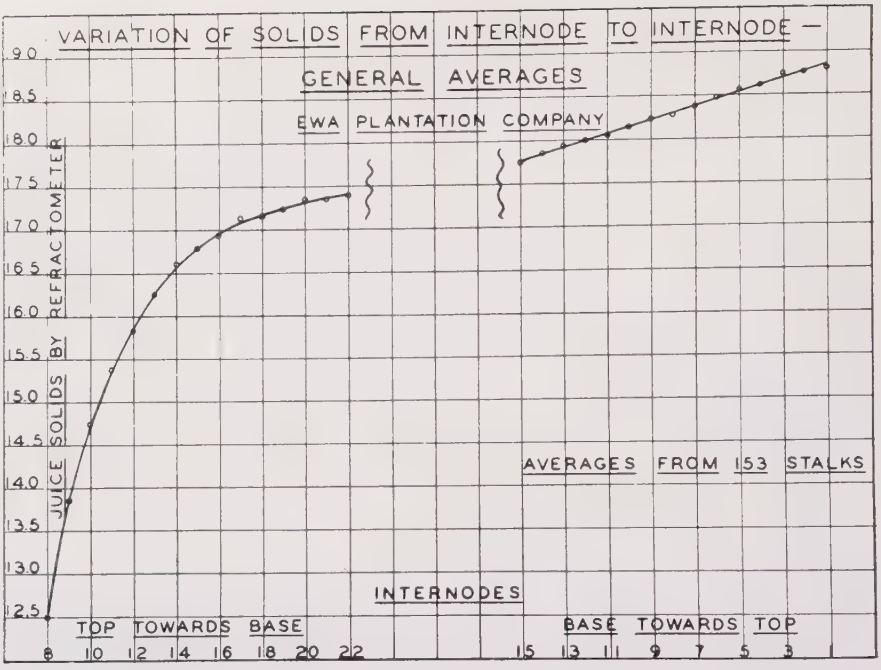


Fig. 3

TABLE V

Comparisons of the Solids of the Twentieth Internodes from the Top and the Tenth from the Bottom of the Stalk With the Average Solids of All Internodes

Average Solids All Internodes			Solids		Diff.: Average to	
Group	Average	No. Averaged	20th Internode from Top	10th Internode from Bottom	20th Internode	10th Internode
14.0 to 15.9	15.05	32	14.89	16.11	+0.16	—1.06
16.0 to 17.9	16.91	51	17.02	17.87	—0.11	—0.96
18.0 to 19.9	18.69	48	18.81	19.52	—0.12	—0.83
20.0 to 21.9	20.66	12	20.73	21.42	—0.07	—0.76

The over-all range in the variation of the average differences is practically the same in both cases. There appears to be no clear advantage from these data in the use of one or the other point of sampling or presumably of any other point, consistently followed between those specified. In order to avoid the possibility of the samplers taking borings near the top, it was decided to limit our work to the tenth internode from the bottom.

Variation of Solids Within Internodes

In order to determine the variation of juice solids within the tenth internode from the bottom, data were collected on five equally spaced points of sampling. A boring was taken at each node and three borings equally spaced between the nodes. The summarized data are as follows:

TABLE VI

Variation of Solids Within Internodes

Group Averaged*	No. Averaged	Upper Node	2	3	4	Lower Node
14.0—15.9	13	15.58	15.25	15.15	15.40	15.98
16.0—17.9	19	16.67	17.16	17.07	17.20	16.89
18.0—19.9	27	18.41	19.21	19.03	19.04	18.39
20.0—21.9	14	19.76	20.74	20.81	20.67	19.99
All	73	17.71	18.25	18.17	18.23	17.88

In all cases except the lowest group where the opposite is true, the nodes yielded appreciably lower solids than the points in the internode. The three points sampled in the internode show a variation close enough to be disregarded in work of this nature.

Method of Sampling: Entirely Through the Stalk or Part Way

Inasmuch as the diameter of cane stalks vary, the question arose as to whether the data might be appreciably influenced by boring entirely through the stalk as against boring only to the depth of the "gun" tip as ordinarily used. The collected data on this point follow, the comparative borings being taken from the same internode.

TABLE VII

Boring Through the Stalk Compared With Boring Part Way as Affected by Stalk Circumference

Group Averaged†	Average Circumference	No. Averaged	Solids of Samples Taken Through Stalk	Solids of Samples Taken To Depth of Tip	Difference
2.0—2.9	2.6	5	21.76	21.74	0.02
3.0—3.9	3.5	20	18.92	18.93	—0.01
4.0—4.9	4.2	27	18.43	18.31	0.12

No differences large enough to be of influence in this method of sampling are to be noted.

Deterioration of Samples

As a necessary step in the development of our technique, studies were attempted on the keeping qualities of the borings. In the first series of data (Table VIII), three borings were taken per internode, solids being determined from one boring immediately, one after 12 hours in the laboratory and the third after 24 hours. Each boring was kept in its individual vial, which was closed with the cork lined screw top provided. In the second series, two samples only were taken from each internode concerned, the comparisons being then between a control and 12 hours in the laboratory, and a control and 24 hours in the laboratory.

* Based on solids of mid-point or No. 3 above.

† Circumference in inches.

TABLE VIII

Effect of Delays on Determination of Solids

	No. Averaged	Solids at Sampling	Solids 12 Hours Later	Solids 24 Hours Later
First Series	42	18.62	18.50	18.69
Second Series (a)	99	18.27	18.12	....
(b)	100	19.37	....	19.36

A more detailed study of the data than is shown in Table VIII indicates a tendency for the solids to decrease slightly in 12 hours followed by slight increase. In any event the differences are small enough to be negligible in this work.

Data on the deterioration of the juices when the borings were collected in numbers and the juice expressed in one operation, follow the outline given above. The averages of 10 comparative tests are reported below:

TABLE IX

Deterioration of Juices from Borings

	At Sampling	12 Hours Later	24 Hours Later
Solids	17.39	17.19	17.23
Pol'n	14.88	14.60	14.42
Purity	85.57	84.93	83.69

The steady decrease in polarization and purity is at once apparent. The purity drop after 24 hours is almost 2 points, which is certainly excessive.

Attempts at using preservatives, including formalin, toluol, ammonium hydroxide and ethyl ether were unsatisfactory. The use of refrigeration was effective, however, as is shown in Table X, which gives the average of 12 tests.

TABLE X

Effect of Refrigeration on Deterioration of Juices from Borings

		Control—	Refrigeration—
	At Sampling	24 Hours Later	24 Hours Later
Brix	17.18	17.03	17.27
Pol'n	14.47	14.05	14.57
Purity	84.23	82.50	84.37

Whenever necessary to delay the analyses of the samples in our work, these were placed on ice immediately on arrival at the laboratory.

Comparison of Solids Determined from Averages of Analyses of Individual Borings and from Numbers of Borings Expressed in One Operation

The amount of laboratory work demanded to obtain the solids of large numbers of individual borings led us to express the juice from a hundred or more borings in the screw press and to analyze this juice for solids and polarization. In order to compare the data obtained from these two procedures, two borings were taken from each internode sampled, one of these being placed in a glass vial and the

other being dropped into a tin box, into which all such borings from a given field location were placed. The summarized data on this point are given below:

TABLE XI

Comparison of Solids Determined from Individual Analyses of Borings and from Expressed Juice

Solids— Groups Averaged*	No. per Group	Solids by Refractometer: Average of Individual Analyses	Expressed Juice	Difference
17.0—17.9	5	17.51	17.57	—0.06
18.0—18.9	11	18.58	18.70	—0.12
19.0—19.9	5	19.41	19.67	—0.26
20.0—20.9	1	20.23	20.17	+0.06
All	22	18.60	18.73	—0.13

Considering the variations found in pre-harvest data, the difference between these two procedures is negligible though the solids of the expressed juices evidently run slightly higher, as a rule, than the average of individual analyses. The saving in time and labor by expressing the juice from a large number of borings at one time as against the observation of hundreds of individual samples, is obvious.

Purity of Juices from Borings Compared With Crusher Juice

At this point, it may be well to summarize our data comparing the purity of the juices expressed from the borings taken with the sampling gun and the purity of crusher juice. Fields immediately previous to harvest were sampled by travelling across the areas according to a designated direction previously indicated on the field maps and taking a boring at the tenth internode from the base, the stalks being selected at random, about every 15 feet. With such data we give the weighted average crusher juice purity at harvest.

TABLE XII

Purity of "Refractometer" Juices Compared With Average Crusher Juices by Fields

Purity Groups Based on Crusher Juice	No. of Fields	Purity of Juices: Crusher	Refractometer	Difference
82.0—82.9	2	82.79	90.03	7.24
83.0—83.9	3	83.70	90.55	6.85
84.0—84.9	5	84.43	91.31	6.88
85.0—85.9	5	85.57	92.09	6.52
86.0—86.9	8	86.51	92.31	5.80
87.0—87.9	5	87.38	91.96	4.58
88.0—88.9	9	88.44	92.29	3.85
89.0—89.9	1	89.33	93.39	4.06
All	38	86.34	91.87	5.53

* Based on averages of "individual"-analyses.

The purities of the juices from the pre-harvested samples are relatively insensible to wide differences in the purities of crusher juices and are evidently of little value in estimating crusher juice purities. For this work, then, we will confine ourselves to a consideration of solids only.

Solids of the Tenth Internode Compared With Sample Mill Juices of Same Stalks; and With Crusher Juices from Experiment Plots

The first step in attempting to apply this method of sampling to practical use was to compare the solids of the tenth joint with the Brix of our sample mill juice. The work was done on samples of five stalks each, each stalk being sampled for the refractometer at the tenth internode from the base and the same stalks then being crushed in the sample mill. Experiment plots were also sampled immediately previous to harvest, 25 borings per watercourse plot, and this compared with the crusher juice samples taken for each plot.

TABLE XIII

Comparison of Solids—"Refractometer" Sampling With Sample Mill and With Crusher Juices

Solids Groups— Based on Refractometer	Refractometer and Sample Mill				Refractometer and Crusher			
	No.	Ref. Solids	Brix	Diff.	No.	Ref. Solids	Brix	Diff.
12.0—12.9	6	12.31	13.27	—0.96				
13.0—13.9	8	13.56	14.00	—0.44	1	13.84	15.13	—1.29
14.0—14.9	21	14.56	14.67	—0.08	2	14.82	15.24	—0.42
15.0—15.9	27	15.50	15.79	—0.29	2	15.32	15.05	+0.27
16.0—16.9	35	16.53	16.33	+0.20	7	16.56	16.85	—0.29
17.0—17.9	30	17.46	17.11	+0.35	15	17.61	17.43	+0.18
18.0—18.9	18	18.55	17.77	+0.78	19	18.43	18.20	+0.23
19.0—19.9	7	19.55	18.81	+0.74	13	19.42	18.85	+0.57
20.0—20.9	6	20.36	19.70	+0.66	7	20.27	19.09	+1.18
21.0—21.9	3	21.41	19.97	+1.44	1	21.16	20.00	+1.16

Apparently a tendency is present for the refractometer solids of the samples to be lower than the Brix of the sample mill juices and of the crusher juices below 16 per cent solids; and to be higher above this. The data are not adequate enough and fluctuate too widely to allow a generalization which can be applied to practical work with any assurance. Rather than attempt to accumulate further data on this point, we proceeded directly to the pre-harvest sampling of fields and to the comparisons between pre-harvest juice and crusher juice analyses by fields.

Pre-Harvest Sampling With Refractometer and Sample Mill Compared

During the crops of 1928 and 1929, a total of 88 fields were sampled, both for the refractometer and for the sample mill. A comparison between the two methods of sampling, according to the established procedure in each case, is thus afforded:

TABLE XIV

Comparison of Crusher Juice Brix and Pre-harvest Solids

Brix Groups Based on Crusher Juice	No. of Fields	Solids Crusher	Solids Sample Mill	Solids Refractometer	Difference: Crusher to: Sample Mill	Difference: Crusher to: Refractometer
16.50—16.99	2	16.90	18.28	17.15	—1.38	—0.25
17.00—17.49	8	17.30	17.96	17.51	—0.66	—0.21
17.50—17.99	13	17.74	17.98	18.00	—0.24	—0.26
18.00—18.49	14	18.34	18.66	18.54	—0.32	—0.20
18.50—18.99	15	18.77	18.87	19.03	—0.10	—0.26
19.00—19.49	21	19.26	18.93	19.25	+0.33	+0.01
19.50—19.99	13	19.67	19.28	19.41	+0.39	+0.26
20.00—20.49	2	20.04	19.68	20.03	+0.36	+0.01
			Group Averages			
16.50—18.99	52	18.10	18.43	18.34	—0.33 ±0.48	—0.24 ±0.39
19.00—20.49	36	19.45	19.09	19.36	+0.36 ±0.43	+0.10 ±0.37
All	88	18.65	18.70	18.75	—0.05 ±0.52	—0.10 ±0.41

There is apparently a break at about 19 Brix, below this point the pre-harvest solids in both methods being higher than the crusher; and above, lower than the crusher. The averages of these two large groups of data are shown in Table XIV. The probable errors of the mean differences are appreciably greater than the differences themselves, indicating that they are of no significance. The considerable fluctuation of the data is, by this means, also apparent.

It was originally intended to estimate the purity of crusher juice by referring the solids determined by refractometer, to the Brix-purity curve for crusher juice. Since the Brix-purity relationship varies with the season of harvest, a detailed application of the method might have been elaborated. It is obvious, however, that fluctuations so large as to show a probable error of about ±0.4, preclude such a study. It will be of some value, however, to compare the pre-harvest procedures on the basis of purity, estimating the crusher juice purity, in the case of the work with the refractometer, by reference to the general Brix-purity relationship of crusher juice as given by our data of the crops of 1924 to 1928, inclusive.

TABLE XV

Brix-Purity Relationship of Crusher Juice

Brix Range	No. Samples	Brix	Pol'n	Purity
14.0—14.4	33	14.22	11.44	80.45
14.5—14.9	72	14.74	11.95	81.07
15.0—15.4	231	15.24	12.46	81.76
15.5—15.9	469	15.73	13.07	83.09
16.0—16.4	1063	16.23	13.58	83.67
16.5—16.9	1770	16.71	14.09	84.32
17.0—17.4	2588	17.21	14.68	85.30
17.5—17.9	3253	17.70	15.18	85.76
18.0—18.4	3611	18.20	15.71	86.32
18.5—18.9	3621	18.70	16.23	86.79
19.0—19.4	2923	19.19	16.70	87.02
19.5—19.9	1930	19.67	17.17	87.29
20.0—20.4	943	20.17	17.58	87.16
20.5—20.9	326	20.64	17.98	87.11
21.0—21.4	112	21.17	18.42	87.01
21.5—21.9	35	21.60	18.71	86.62

Interpreting the pre-harvest refractometer solids of the 88 fields previously considered, we obtain the following purity data:

TABLE XVI

Comparison of Crusher Juice and Pre-harvest Purities

Purity Groups Based on Crusher Juice	No. of Fields	Purity			Differences—Crusher to:		
		Crusher	Sample Mill	Refracto-meter	Sample Mill	Refractometer	Corrected Sample Mill
82.0—82.9	3	82.85	85.76	86.44	—2.91	—3.59	+0.06
83.0—83.9	3	83.70	88.28	86.91	—4.58	—3.21	—1.61
84.0—84.9	6	84.46	88.49	86.82	—4.03	—2.36	—1.06
85.0—85.9	11	85.53	89.61	86.16	—4.08	—0.63	—1.11
86.0—86.9	24	86.49	89.31	86.45	—2.82	+0.04	+0.15
87.0—87.9	20	87.40	90.00	86.60	—2.60	+0.80	+0.37
88.0—88.9	19	88.45	90.77	86.98	—2.32	+1.47	+0.65
89.0—89.9	2	89.50	92.61	87.16	—3.11	+2.34	—0.14
Averages	88	86.71	89.68	86.22	—2.97 ±0.84	+0.49 ±1.06	

As practically all of the estimated "refractometer" purities lie between 86 and 87 irrespective of the purity of crusher juice, this procedure is obviously of little value in pre-harvest estimates of crusher juice purities. The difference of 2.97 between the purities of the sample mill and of the crusher juices is significant as shown by the probable error. Applying this difference as a correction, the data of the sample mill still remains far from accurate.

The probable errors of the differences between the Brix of crusher juice and the Brix of sample mill juice as compared with refractometer solids of Table XIV indicate that the refractometer procedure is not inferior to the sample mill in so far as the estimation of the Brix of crusher juice is concerned. Inasmuch as the Brix of crusher juice is in general correlated with the purity of crusher juice, the determination of the total solids of pre-harvest samples by the refractometer offers one method of following the ripening of cane. Some typical examples of the use of this procedure in comparison with the use of the sample mill follow:

TABLE XVII

Pre-harvest Sampling by Refractometer and Sample Mill Compared

Field	Refractometer Date	No.	Av. Solids	Sample Mill Brix	Purity	Q. R.	Date	Crusher at Harvest Brix	Purity	Q. R.	Date
W	Jan. 12	150	15.18	16.10	86.21	9.80	Jan. 13				
	Feb. 23	127	16.36	18.00	90.28	8.10	Feb. 21				
	Mar. 26	148	17.54	18.13	90.57	8.00	Mar. 28				
	April 30	149	18.28	19.82	90.01	7.39	April 30	17.89	87.03	8.67	May 3–16
X	Jan. 18	149	18.03	17.53	88.41	8.64	Jan. 18				
	Mar. 6	297	18.80	18.94	91.21	7.60	Mar. 6				
	Mar. 28	150	19.39	19.35	91.16	7.41	Mar. 28	19.43	88.99	7.69	April 5–9
Y	Jan. 14	150	14.14	12.73	69.36	18.80	Jan. 16				
	Mar. 3	149	16.91	16.47	85.61	9.69	Feb. 25				
	April 5	122	18.76	16.97	87.39	9.08	Mar. 31				
	May 5	137	18.52	19.20	90.26	7.59	May 14				
	June 16	150	19.30	18.93	89.65	7.79	June 16				
	July 10	150	19.19	18.30	89.78	8.04	July 10				
	Aug. 6	101	19.33	20.13	90.56	7.20	Aug. 6				
	Aug. 24	104	20.14	18.77	88.28	8.07	Aug. 24	19.61	86.69	7.97	Aug. 27–Sept. 1
Z	Jan. 17	122	14.89	15.63	82.53	10.90	Jan. 16				
	Mar. 5	153	16.73	15.83	84.46	10.33	Feb. 25				
	April 4	123	17.71	17.73	88.16	8.56	Mar. 31				
	May 7	161	18.76	19.37	90.50	7.49	May 14				
	June 16	172	18.58	19.67	91.00	7.31	June 15				
	July 10	147	19.60	20.33	91.34	7.03	July 10				
	July 21	198	19.58	18.63	87.33	8.28	July 21	19.06	86.73	8.19	July 23–28

It is, of course, desirable to obtain some idea of the relative purity in pre-harvest sampling. If, however, the purity data are not desired, this procedure becomes considerably less expensive. Furthermore, inexpensive pycnometers may be used in place of the refractometer, expressing the juice from one or two hundred sample lots in one operation.

Frequency Data

As a part of this work, it was desired to obtain fairly comprehensive data on the solids characteristics of the stalk population of fields approaching maturity in order to gain some idea of the problem involved in the pre-harvest sampling of cane. Such data should afford some foundation for further work on the development of pre-harvest sampling technique.

In Table XVIII, the sampling data from over 22,000 individual stalks are assembled. These data were available from the sampling of the fields of the 1929 crop through the month of March. The averages are given according to the months of harvest and of sampling. The data of individual fields falling either above or below the group average by one or two classes have been adjusted by moving the frequency data up or down one or two classes. Thus, if the group average fell into the 17.0 to 17.9 class and the field in question showed an average falling into this same class, the data were used without modification. If, however, the field average fell into the next lower class or 16.0 to 16.9 solids, then the entire frequency of that field was moved up one class for the summary. This procedure adjusts the data to a distribution about the group mean but does not destroy the relative shape of the curve.

TABLE XVIII

Frequency Data in Per Cent by Months of Sampling and of Harvest—Solids of the Tenth Internode by Refractometer

Total	Group Averages	Months of Sampling	2.0–2.9	3.0–3.9	4.0–4.9	5.0–5.9	6.0–6.9	7.0–7.9	8.0–8.9	9.0–9.9	10.0–10.9	11.0–11.9	12.0–12.9	13.0–13.9	14.0–14.9	15.0–15.9	16.0–16.9	17.0–17.9	18.0–18.9	19.0–19.9	20.0–20.9	21.0–21.9	22.0–22.9	23.0–23.9	24.0–24.9
Fields Harvested December																									
1178	18.62	May–June	....	....	....	....	....	....	....	....	....	....	0.30	1.01	2.78	5.56	8.43	17.54	21.31	20.83	13.67	6.77	1.45	0.57	
1057	17.70	August	....	....	....	....	....	....	....	0.21	0.90	1.07	3.53	3.21	5.25	9.81	11.36	17.49	18.88	16.72	8.11	2.54	0.85	0.08	
643	17.94	September	....	....	....	....	....	....	....	0.23	1.70	1.79	2.72	2.87	6.17	7.38	13.40	15.99	21.14	16.79	8.06	1.75	....	...	
547	17.61	October	....	....	....	....	....	....	0.31	0.66	1.18	1.15	3.38	3.68	4.80	8.98	12.72	17.11	16.18	17.60	8.12	4.01	0.14	....	....
843	17.80	November	....	....	....	....	....	0.16	0.29	0.59	1.77	1.20	1.30	2.41	5.39	5.94	14.16	15.93	18.89	20.35	7.95	2.09	0.79	0.60	0.20
914	18.27	December	....	....	....	0.10	0.10	0.11	0.39	0.28	0.21	0.38	0.48	1.36	2.56	4.49	9.41	15.10	19.93	20.58	16.31	6.65	1.44	0.11	
5182																									
Fields Harvested January																									
1044	17.00	May–June	....	....	....	....	....	....	....	....	0.34	0.52	0.81	2.02	6.02	8.94	17.51	19.85	17.88	14.57	7.51	3.21	0.73	0.08	
750	16.97	August	....	....	....	....	....	....	....	....	0.74	1.14	1.54	3.68	7.19	9.11	12.93	19.20	17.48	14.10	8.17	3.72	0.98		
853	17.02	September	....	....	....	....	....	....	....	0.81	1.88	2.17	3.35	2.37	6.29	9.41	12.46	17.86	18.39	12.59	8.46	3.64	0.32	...	
669	17.10	Oct.–Nov.	....	....	....	....	....	....	0.77	0.80	1.20	1.81	3.19	2.22	5.79	6.18	12.83	13.98	16.31	16.63	11.91	5.78	0.30	0.31	
1784	17.25	Dec.–Jan.	....	0.07	0.12	....	0.29	0.32	0.35	0.28	0.84	1.47	1.57	3.09	3.84	6.10	14.67	20.59	22.33	15.93	5.75	1.92	0.40	0.07	
5100																									
Fields Harvested February																									
1187	16.82	May–June	....	....	....	....	....	....	....	....	0.46	0.22	1.20	1.82	4.92	13.85	17.57	21.68	19.47	11.22	5.82	1.38	0.38		
899	16.97	August	....	....	....	....	....	....	....	....	0.16	1.17	1.77	4.15	5.68	9.08	16.02	19.15	15.04	15.40	8.40	3.30	0.67	....	
1170	17.44	Sept.–Oct.	....	....	....	....	....	....	0.06	0.39	1.21	2.83	2.18	3.09	4.55	6.13	11.47	16.80	21.16	15.42	8.80	5.16	0.69	0.05	
750	17.41	December	....	....	....	....	0.12	0.11	0.22	....	0.42	0.97	1.61	1.76	4.01	7.55	13.75	21.41	22.01	13.91	7.16	4.15	0.72	0.12	
938	17.43	January	....	....	0.12	0.09	0.25	0.08	0.23	0.81	0.68	0.88	1.81	2.98	4.92	10.21	13.38	21.20	19.96	13.53	7.43	1.36	0.09		
776	18.57	February	....	....	....	0.10	0.13	0.56	0.28	0.11	0.40	0.84	0.55	1.88	2.67	4.02	8.05	12.70	18.48	25.07	16.59	6.40	1.16		
5720																									
Fields Harvested March and April																									
1044	17.62	May–June	....	....	....	....	....	....	....	....	....	....	0.98	1.94	5.92	9.61	19.34	19.57	16.87	14.14	7.44	3.33	0.87	...	
812	16.97	August	....	....	....	....	....	....	....	0.10	0.41	1.26	2.30	3.94	4.85	10.00	12.99	14.25	21.34	16.98	6.77	3.78	0.83	0.20	
1050	16.66	September	....	....	....	....	....	....	0.52	2.30	2.64	3.85	3.59	5.21	7.14	10.26	12.17	18.54	19.90	10.24	2.87	0.55	0.23		
864	16.88	January	0.08	....	0.08	0.50	0.50	0.35	1.12	1.25	1.49	2.23	2.39	5.26	10.55	12.75	14.30	18.21	15.77	8.49	2.62	0.55	....	....	....
1196	17.63	February	0.06	0.10	0.15	0.19	0.25	0.10	0.24	0.89	0.52	1.15	2.67	2.99	5.36	8.08	13.85	17.98	20.21	12.92	8.37	3.08	0.84	....	....
1236	18.71	Mar.–April	....	....	0.06	....	0.05	0.13	0.41	0.49	0.30	0.84	0.92	1.16	2.31	4.02	7.56	11.14	15.30	20.57	20.10	11.34	3.08	0.17	0.05
6202																									

Total 22,204

A surprisingly large variation in the solids of individual stalks at the tenth internode from the base is evident (Fig. 4) and offers some explanation for the difficulties encountered in obtaining accurate pre-harvest samples. For this reason, too, we rely on the trend of our sampling data from month to month rather than accept the data as they stand at any one sampling.

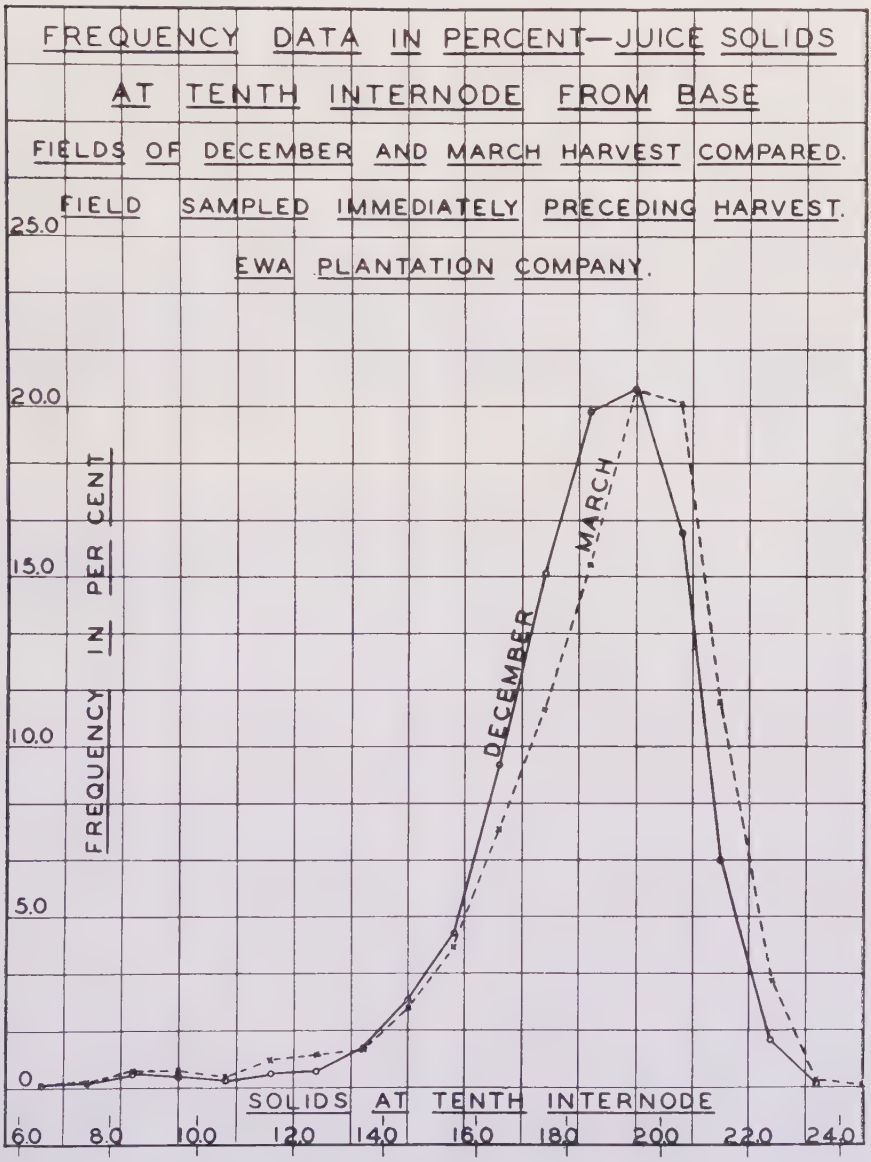


Fig. 4

TABLE XIX

Variation of Solids by Refractometer of Adjacent Stools—H 109

		Line 1					Line 2			
	Stool No.	No. Stalks*	Average†	Highest	Lowest	Suckers	No. Stalks*	Average†	Highest	Lowest	Suckers
Field 10A	1	10	16.45	19.0	15.0	..	3	15.27	15.6	15.0	.....
June 27, 1929	2	2	13.25	16.5	10.0	..	6	13.38	16.6	11.6	.....
Age 11.9 Months	3	2	13.35	13.9	12.8	..	6	17.00	18.0	16.0	.....
Plant	4	6	15.78	17.0	14.6	..	2	16.50	17.5	15.5	.....
	5	9	14.41	18.0	11.1	9.8	3	17.40	18.0	16.4	.....
	6	2	13.85	14.1	13.6	..	2	14.75	16.2	13.3	.....
	7	4	15.15	16.5	13.7	..	6	17.00	19.2	13.0	.....
	8	2	14.10	15.3	12.9	..	10	14.87	17.6	11.1	6.9
	9	7	14.74	19.1	11.0	..	10	13.27	15.2	10.0	4.0
	10	4	17.23	19.3	13.9	..	9	15.11	17.5	12.4	.....
	11	6	14.70	17.0	12.7	..	2	12.40	13.0	11.8	.....
	12	7	16.16	20.1	12.2	..	7	13.64	15.3	11.6	8.4 & 9.7
	13	10	18.17	20.6	16.2	..	5	13.80	14.5	13.1	.....
	14	2	16.95	17.3	16.6	..	2	12.40	12.4	12.4	.....
	15	13	17.93	20.2	15.4	7.9	6	11.90	12.9	11.0	3.2, 6.4 & 7.3
	16	10	15.93	17.5	13.9	..	2	10.80	....	....	7.8
	17	4	16.30	17.9	14.7	..	6	13.97	16.6	10.6	.....
	18	9	15.69	17.9	13.7	..	4	14.25	14.5	14.0	5.5 & 9.1
	19	5	15.38	17.0	12.9	..	10	14.88	17.0	13.8	.....
	20	1	16.00	....	....	..	9	12.39	14.6	10.6	4.0 & 9.0
	21	9	16.54	18.5	13.9	..	4	12.23	13.8	11.2	.....
	22	6	14.92	18.0	11.9	6.5	5	12.88	14.1	11.9	5.4
	23	3	15.40	16.7	13.3	..	4	13.10	14.3	11.0	7.6
	24	..	....	....	....	..	3	13.37	14.1	12.4	.....

* Including suckers.
† Excluding suckers.

This variation is also shown in a small test on the variation between adjacent stools in plant cane about a year old. (Table XIX.) The stalks were sampled at the tenth internode consecutively in line. The variations between the stools are so great as to render the use of this sampling procedure in variety work of doubtful application under local conditions.

Summary

1. A pre-harvest sampling technique involving the use of the refractometer has been attempted.

2. This technique has been compared with the usual sample mill procedure in field trials. For the estimation of crusher juice Brix, the refractometer method of sampling is not inferior to that of the sample mill. The method is not applicable to the estimation of crusher juice purity.

3. The progress of cane ripening can be followed in a general way through pre-harvest sampling for the determination of solids only.

4. Frequency curves indicate a great variation in the total solids of the stalk population of fields approaching maturity. This is true also of individual stools in plant cane about a year old.

Pollen Studies II

Germination of Sugar Cane Pollen in Culture Media

By D. M. Weller

In 1926, there appeared in *The Hawaiian Planters' Record* (4) a paper entitled "Progress Report of Sugar Cane Pollen Studies." In that paper was given a quantitative method of germinating sugar cane pollen by limiting the factors conducive to pollen germination to two factors, viz., relative humidity and temperature. A brief review was also given there of the methods used by different investigators from different parts of the sugar cane world whereby the determining of viability of sugar cane pollen had been attempted. It is apparent from that review that, in the minds of these investigators, the criterion of viability was the actual formation of pollen tubes by the pollen grains. Also the desirability of securing quantitative data was suggested.

A statement occurs there to the effect that trials at this Station in recent years to germinate cane pollen in culture media were unsuccessful. With the thought that it would be interesting to know just what these trials were, a careful search of the project files of this Station was made. Two reports were found, the first one by Dr. L. O. Kunkel and the second one by C. C. Barnum.

Review of Attempts to Germinate Sugar Cane Pollen at This Station

The following is taken from the *Monthly Letter* of this Experiment Station for the period from November, 1922, to January, 1923:

> Sugar cane pollen differs from that of many plants in that it will not germinate on artificial media. Dr. Kunkel reports experiments as follows:
>
> During the past month, an effort was made to germinate pollen grains of sugar cane in artificial media. Fresh pollen of the varieties D 1135 and H 109 was dusted into plates containing the following media: nutrient agar, dextrose agar, cane sugar agar, potato agar, bean agar, oat agar, synthetic agar, Biejerinck agar and water agar. The test was repeated several different times, but in no case did a single grain germinate. With the exception of synthetic agar, the pollen remained plump and in apparently good condition on all of the different media. Unsuccessful germination tests were also made in hanging drops of water, and in 2 per cent sugar solution. Germinations were, however, obtained by dusting fresh pollen onto stigmas of sugar cane and papaya. Because of the large size and smooth surfaces they possess, papaya stigmas are especially well suited for germinating cane pollen. Some germinating grains were scraped from papaya stigmas and placed in iodine solution in permanent mounts.
>
> A considerable quantity of D 1135 pollen was collected from day to day and brushed onto the stigmas of Uba, growing at the Federal Experiment Station. It is hoped that this will give rise to D 1135-Uba seedlings. Anthers of Uba, so far as examined, do not produce fertile pollen. The ovaries seem to be normal.

In the *Monthly Letter* for December, 1924, H. Atherton Lee writes as follows:

> The best tests of the viability of pollen are the actual formation of pollen germ tubes, and such germination was secured fairly uniformly on Petri dishes in which drops of 5

per cent glucose solution were placed. Germination did not take place in the drops of the glucose solution, but in the condensed moisture on the plates between the drops.

Under date of March 20, 1926, Mr. Barnum reports the results of his trials to germinate cane pollen on agar plates. These trials were made during November and December of 1924, and are the trials mentioned above by Mr. Lee.

Mr. Barnum says in his report:

An attempt to germinate the pollen in Petri dishes on the surfaces of various concentrations of agar proved unsuccessful. The pollen grains usually remained turgid and of normal size during periods of three to six or more days on all sterile poured plates of agar. No germination was noted. The plates were maintained at room temperature and the concentrations of the agar jelly were as follows: agar and water only in the following percentages: 1.50, 2.00, 3.00, 4.00 and 5.00 per cent; nutrient agar of 30 per cent, and nutrient agar, 3.00 per cent plus 2.00 per cent sucrose. For these germination studies on agar both H 109 and D 1135 tassels were shaken over separate plates at 6, 7, 8, 9 A. M. on November 8, November 9, and November 11, 1924. The tassels used for this study were kept in sulphurous acid solutions in the new greenhouse.

A little further on in Mr. Barnum's report occurs a record of attempts to germinate cane pollen in varying concentrations of sugar solutions. He writes:

An attempt to germinate pollen grains on microscope slides on which thick cane sugar syrup had been partially dried in separate drops was attempted. Pollen was dusted over such slides and they were then placed in moist chambers. Pollen taken at 11:30 A. M. November 18, failed to germinate both on the plate surface and in the syrup. Further studies were made using sucrose and dextrose syrups separately. Negative results were obtained in cane sugar or sucrose, but slight germination was noted in dextrose.

At this point, no explanation of the failures contained in these two reports will be attempted but a comment upon them will be offered further on.

Purpose of the Present Studies

It is the practice in the cane-breeding work in Hawaii to bring together male and female tassels by placing them with their cut ends in vessels of sulphurous acid. It is desirous to know in this work: (1) what effect different concentrations of sulphurous and other acids (including the ones now used as common practice) have on the percentage of viability of pollen produced by tassels so treated, (2) how the percentage of viability of pollen from cut tassels compares with that of pollen from growing tassels on successive days after their initial pollen shed (the cut tassels to be cut on the day of their first flowering), (3) how long pollen grains remain viable after being shed, (4) how effective methods for preserving pollen in viable form (so that early flowering varieties may be crossed with late flowering varieties) may be in case such methods are developed, and (5) how the percentages of viability of the different varieties used compare.

Only a standardized method of germinating cane pollen can answer these questions.

The purpose of these studies was, therefore, to standardize a method for the securing of quantitative data in regard to the above five points.

Methods and Materials

In 1927, Dutt and Ayyar (3) reported the obtaining of high percentages of germination of pollen from a great number of cane varieties. This method consisted of using a culture medium of 26 per cent sucrose plus 0.7 per cent agar as "hanging drops" in culture slides. A thin layer of the medium was spread on a coverslip and dusted with pollen. This coverslip was then inverted and sealed with vaseline over the cavity in the culture slide. Drops of the medium were placed in the bottom of the cavity of the hollow ground slide to prevent drying of the medium on the coverslip. Later the percentage of germination of the pollen was determined by examining these coverslips under the microscope.

This method of Dutt and Ayyar was tried using a number of media because it was surmised that under conditions which prevail in Hawaii and with pollen from the Hawaiian varieties of cane a higher or lower concentration of sugar or agar in the medium might be necessary for successful germination, or that different concentrations might be required for pollen from different varieties of cane. The contents of these media are shown in Table I:

TABLE I

Medium No.	Agar Grams	Sucrose Grams	Water Ml	Agar	:	Sucrose
1	0.17	24	100	1	:	141.1
2	0.35	25	100	1	:	71.4
3	0.70	26	100	1	:	37.1
4	1.00	27	100	1	:	27.0
5	1.30	28	100	1	:	21.5
6	1.70	29	100	1	:	17.0
7	2.00	30	100	1	:	15.0

Commercial granulated sugar, "Difco" Standardized, a Bacto-agar made by the Digestive Ferment Company of Detroit, Michigan, and distilled water were used in making up these media.

Except where stated to the contrary, the pollen used in these tests was taken from tassels which had been cut the previous day and kept over night with their cut ends in an approximate 0.03 per cent solution of sulphurous acid. In order to minimize the influence of variation of pollen from single tassels, three tassels of the same variety were used at a time so that the pollen used in the tests was a composite sample rather than pollen from a single tassel. Only tassels cut the previous day were used because it has been shown that the percentage of viable pollen from tassels kept in sulphurous acid decreases very rapidly after the tassels are cut (4).

Results

Using the above methods and materials ten preliminary tests were made with the results shown in Table II:

Table II

Showing the percentages of germination of pollen from several varieties of cane obtained with different media (shown in Table I) in hanging drops in culture slides at room temperature.

Medium No.	U. S. 4163	D 1135	U. S. 4163 (1)	S. W. 3 (2)	28- 4363 (2)	20 S 16 (2)	Maka- weli 3	26 Q 2079	H- 27 (2)	D 1135
1	0.0	0.0	0.0	0.0	3.7	0.0	10.0	0.0	0.0	0.0
2	5.2	0.0	12.5	0.0	0.0	0.0	5.0	0.0	4.3	0.0
3	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	7.5	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

(1) Tassels cut two days previously.

(2) Pollen taken from tassels on the same day they were cut.

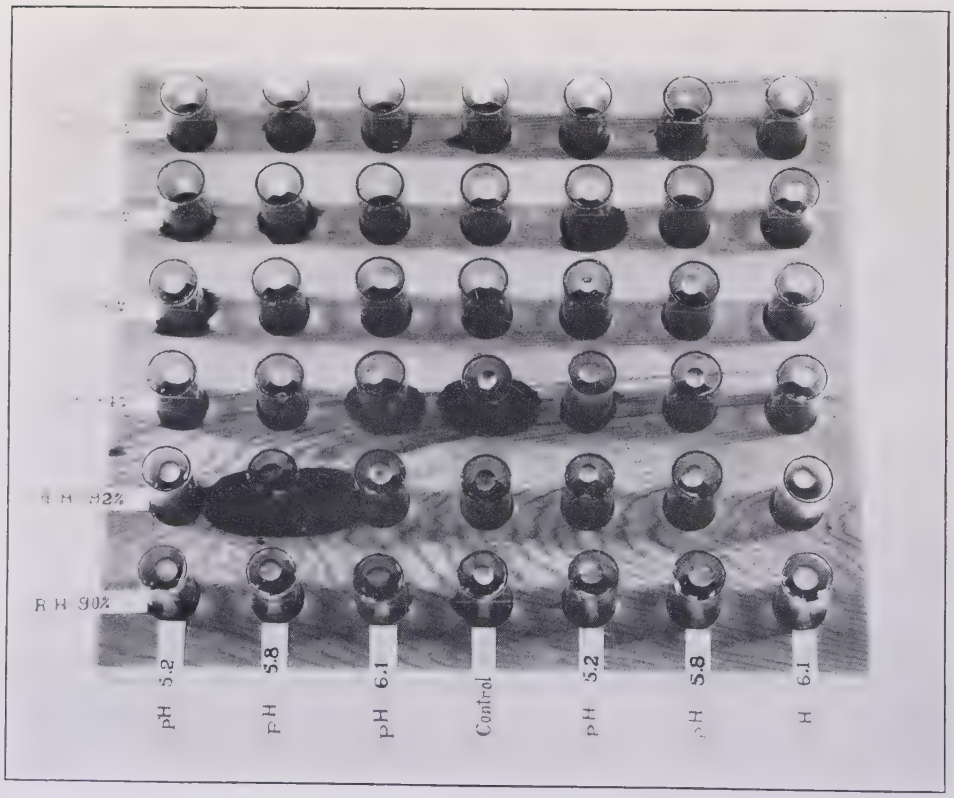


Fig. 1. Showing vials on which are sealed cover glasses with hanging drops of culture media on them. The relative humidities of the air surrounding these drops of culture media are controlled by solutions of sulphuric acid of different concentrations in the vials. Such boards holding the vials were substituted for the shelves of constant temperature ovens operating at different temperatures.

These data seemed to indicate that medium No. 2 held greater possibilities than the others and was, therefore, after several more preliminary tests recorded below, used in future experiments.

Method Using Sulphuric Acid Solutions in Sealed Chambers to Control Relative Humidity

In the next test, all of the media of Table I were used and the factor of relative humidity was introduced. Vials with ground tops were partly filled with different concentrations of sulphuric acid. Drops of agar were placed on coverslips and dusted with pollen. These coverslips were then inverted and sealed with vaseline on the vials containing the sulphuric acid solutions. In each sealed chamber the air over the sulphuric acid solutions had a constant relative humidity dependent upon the concentration of the sulphuric acid solution in that vial and the temperature at which it was maintained (5). (See Fig. 1.) Coverslips without drops of agar were also used, the pollen simply being caught on the dry glass and treated in the same way as those which had drops of agar on them. These are called "controls."

The sulphuric acid solutions were of such concentrations that the relative humidities of the air in the sealed chambers above them were 100, 98, 96, 94, 92 and 90 per cent.

This method was used with pollen from the variety H 27 with the results shown in Table III:

Table III

Showing percentages of germination obtained with H 27 pollen in the media shown in Table I with the constant relative humidity indicated and at room temperature.

Medium No.	Relative Humidity 100%	98%	96%	94%
1	0.0	0.0	0.0	0.0
2	2.1	4.0	5.8	3.8
3	0.0	1.2	4.5	0.5
4	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0
Control	0.8	0.0	0.0	0.0

Here, again, medium No. 2 gave the highest per cent of germination (5.8 per cent) and, at this temperature, 96 per cent was the optimum relative humidity. The percentage of germination in the culture media significantly exceeded that of the controls.

In the next test, the factor of relative humidity was kept in operation, and the factors of temperature and pH values of the media were introduced. This test was run with vials containing sulphuric acid as described in the preceding one. Because the work of 1925 (4) indicated that 22.0° C. was the optimum tempera-

ture, the vials were placed in an incubator at that temperature. Medium No. 2 was used and the pH values were adjusted to 5.2, 5.4, 6.2, 6.9 and 7.7. Relative humidities of 100, 98, 96 and 94 per cent were maintained as before. Controls were also run. Pollen from the variety H 456 was used. The result of this test was that not a single germination occurred. Two controls for each humidity also showed negative germination. We are at a loss for an explanation of this negative germination (unless it were tassel variation) for tests with pollen from this variety later on in the season at 20.0° C., and 23.0° C. resulted in germinations as high as 5.5 and 8.8 per cent respectively.

This test was repeated using again pollen from H 456 with both medium No. 2 and medium No. 3, but at 24.5° C. These results are shown in Table IV:

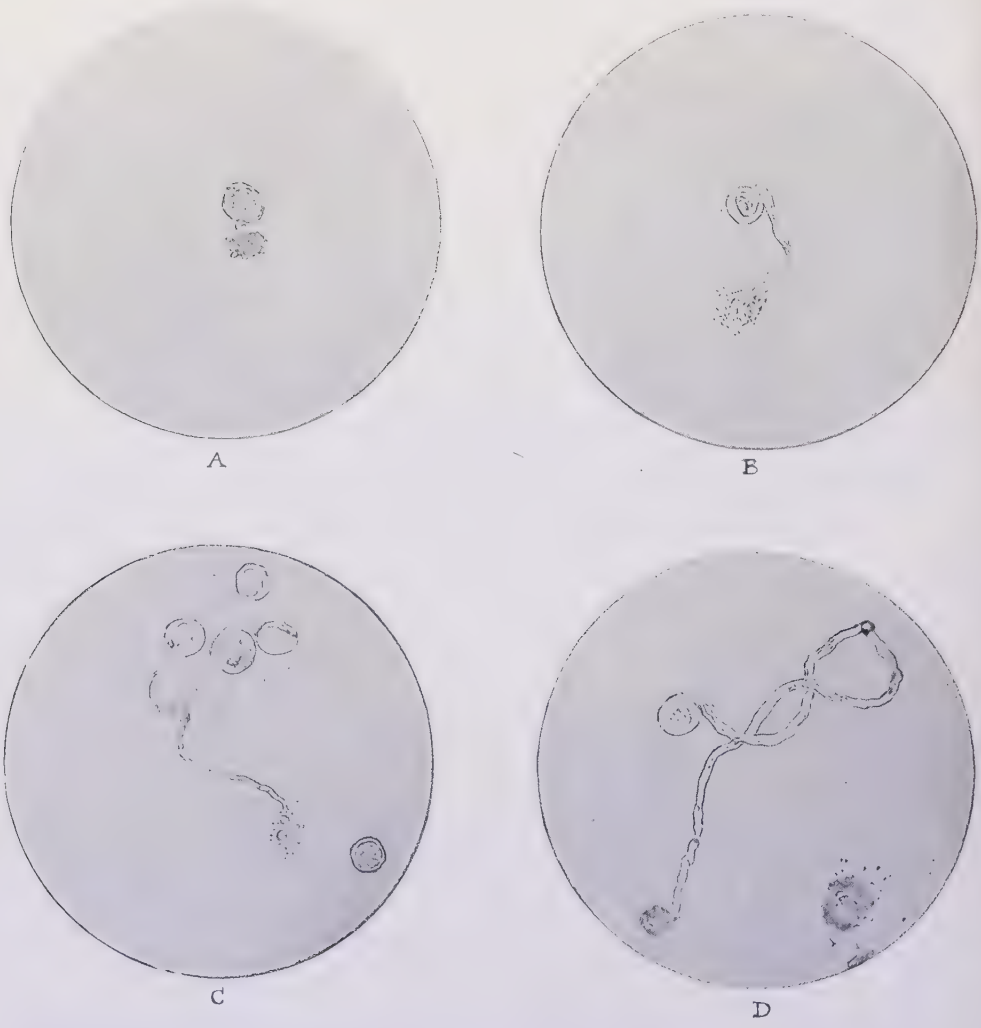


Fig. 2. Showing pollen grains of the variety H 456 germinating at a relative humidity of 96 per cent, in medium No. 2 with a pH value of 5.2, and at 27.0° C. (A), at 25.5° C. (B), at 24.5° C. (C), and at 23.0° C. (D). x140.

These data seemed to indicate that medium No. 2 held greater possibilities than the others and was, therefore, after several more preliminary tests recorded below, used in future experiments.

Method Using Sulphuric Acid Solutions in Sealed Chambers to Control Relative Humidity

In the next test, all of the media of Table I were used and the factor of relative humidity was introduced. Vials with ground tops were partly filled with different concentrations of sulphuric acid. Drops of agar were placed on coverslips and dusted with pollen. These coverslips were then inverted and sealed with vaseline on the vials containing the sulphuric acid solutions. In each sealed chamber the air over the sulphuric acid solutions had a constant relative humidity dependent upon the concentration of the sulphuric acid solution in that vial and the temperature at which it was maintained (5). (See Fig. 1.) Coverslips without drops of agar were also used, the pollen simply being caught on the dry glass and treated in the same way as those which had drops of agar on them. These are called "controls."

The sulphuric acid solutions were of such concentrations that the relative humidities of the air in the sealed chambers above them were 100, 98, 96, 94, 92 and 90 per cent.

This method was used with pollen from the variety H 27 with the results shown in Table III:

Table III

Showing percentages of germination obtained with H 27 pollen in the media shown in Table I with the constant relative humidity indicated and at room temperature.

Medium No.	Relative Humidity 100%	98%	96%	94%
1	0.0	0.0	0.0	0.0
2	2.1	4.0	5.8	3.8
3	0.0	1.2	4.5	0.5
4	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0
Control	0.8	0.0	0.0	0.0

Here, again, medium No. 2 gave the highest per cent of germination (5.8 per cent) and, at this temperature, 96 per cent was the optimum relative humidity. The percentage of germination in the culture media significantly exceeded that of the controls.

In the next test, the factor of relative humidity was kept in operation, and the factors of temperature and pH values of the media were introduced. This test was run with vials containing sulphuric acid as described in the preceding one. Because the work of 1925 (4) indicated that 22.0° C. was the optimum tempera-

ture, the vials were placed in an incubator at that temperature. Medium No. 2 was used and the pH values were adjusted to 5.2, 5.4, 6.2, 6.9 and 7.7. Relative humidities of 100, 98, 96 and 94 per cent were maintained as before. Controls were also run. Pollen from the variety H 456 was used. The result of this test was that not a single germination occurred. Two controls for each humidity also showed negative germination. We are at a loss for an explanation of this negative germination (unless it were tassel variation) for tests with pollen from this variety later on in the season at 20.0° C., and 23.0° C. resulted in germinations as high as 5.5 and 8.8 per cent respectively.

This test was repeated using again pollen from H 456 with both medium No. 2 and medium No. 3, but at 24.5° C. These results are shown in Table IV:

Fig. 2. Showing pollen grains of the variety H 456 germinating at a relative humidity of 96 per cent, in medium No. 2 with a pH value of 5.2, and at 27.0° C. (A), at 25.5° C. (B), at 24.5° C. (C), and at 23.0° C. (D). x140.

Relative Humidity

	100%		98%		96%		94%	
pH	M 2	M 3	M 2	M 3	M 2	M 3	M 2	M 3
4.7 (1)	0.0	0.2	0.0	1.7	0.0	0.8	0.8	0.7
5.2	1.2	0.0	1.7	0.0	3.4	0.6	0.6	0.2
5.8	0.5	0.0	0.6	1.8	0.6	1.3	1.3	2.9
6.1	0.0	2.8	2.0	0.6	0.8	0.0	0.0	0.2
6.4 (2)	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0
Control (3)	0.0		0.0		0.0		0.0	

(1) This pH for medium No. 3 was 4.5.
(2) This pH for medium No. 3 was 6.5.
(3) Average of 4 vials for each relative humidity.

From data in Table V, it is seen that again the maximum germination occurred in medium No. 2 at a pH value of 5.2 and at 96 per cent relative humidity. This was 3.4 per cent while at 24.5° C. it was 5.5 per cent. The maximum for medium No. 3 was 2.9 per cent occurring at a relative humidity of 94 per cent and at a pH value of 5.8. (Fig. 2, B.)

Table VI

Showing the percentages of germination on media No. 2 and No. 3 of H 456 pollen at 27.0° C. and at the pH values and relative humidities indicated.

Relative Humidity

	100%		98%		96%		94%	
pH	M 2	M 3	M 2	M 3	M 2	M 3	M 2	M 3
4.7 (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5.2	0.8	0.0	0.0	0.0	0.0	0.0	0.0	
5.8	0.0	0.0	0.0	0.5	0.0	0.0	0.0	
6.1	0.0	0.0	0.3	0.0	0.0	0.0	0.0	
6.4 (2)	0.0	0.0	0.0	0.3	0.0	0.3	0.0	
Control (3)	0.1		0.0		0.4		0.0	

(1) pH 4.5 for medium No. 3.
(2) pH 6.5 for medium No. 3.
(3) Average of 4 vials for each relative humidity.

At this temperature the maximum per cent of germination for medium No. 2 was 0.8 per cent occurring again at a pH value of 5.2 but at 100 per cent relative humidity; for medium No. 3 it was 0.5 per cent occurring at 98 per cent relative humidity and at a pH value of 5.8. These percentages are not only less than those obtained at 24.5° C. but are also less than those obtained at 25.5° C. (Fig. 2, A.)

The results of these three tests (Tables IV, V, VI) indicate that, for pollen of the variety H 456, the optimum medium is medium No. 2 (shown in Table I); that the optimum pH value of the medium is near 5.2; that the optimum relative humidity is near 96 per cent, and that the percentage of germination decreased as the temperature was increased from 24.5° C. to 25.5° C. and 27.0° C. successively. Because the maximum germination under these conditions was but 5.5 per cent,

it was thought that this percentage might be increased by the action of some enzyme or combination of enzymes. It was planned, therefore, to try certain amylolytic enzymes and later some of the proteolytic enzymes.

Trials With Diastase

A series of experiments was next run, using medium No. 2 alone, and with sputum added, at the rate of 7 ml. to 375 ml. of agar. This latter medium was designated as M2S. The relative humidities, 100, 98, 96 and 94 per cent were maintained as before. The pH values of the two media (M 2 and M 2 S) were adjusted to 5.2, 5.8 and 6.1. Pollen from the varieties H 456 and D 1135 was used. The culture slide method described above was run along with them.

The results of the first of these experiments are shown in Table VII:

Table VII

Showing the effect on the percentages of germination of H 456 pollen of diastase added to medium No. 2 in the form of sputum, at the indicated pH values and relative humidities, and at 27.0° C.

	pH						
	5.2		5.8		6.1		
R. H.	M 2	M 2 S	M 2	M 2 S	M 2	M 2 S	Control (1)
100%	0.0	0.0	0.0	0.0	0.0	3.2	0.0
98%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
96%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
94%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cult. Slds.	0.0	0.0	0.0	0.0	0.0	0.0	0.0

(1) Average of two vials for each humidity.

There was a slight indication here of beneficial effect of the diastase action of the sputum, for 3.2 per cent occurred in the medium to which the sputum was added while in that to which none was added no germinations occurred. The data of Table VI also showed that at 27.0° C. the per cent of germination of this pollen was very low, viz., 0.8 per cent.

The results of a similar test run with pollen from H 456 and from D 1135 at 26.0° C. are shown in Table VIII.

Table VIII

Showing the relative percentages of germination of pollen from the varieties H 456 and D 1135 in media with and without sputum at the indicated relative humidities and pH values and at 26.0° C.

pH

	5.2				5.8				6.1				
	M 2		M 2 S		M 2		M 2 S		M 2		M 2 S		
R. H.	H456	D1135	H456	D1135	H456	D1135	H456	D1135	H456	D1135	H456	D1135	Control
100%	0.8	9.0	0.5	0.0	3.1	5.4	0.0	0.0	1.2	0.0	0.0	12.5	0.0
98	0.0	2.1	0.0	6.2	0.4	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0
96	0.0	0.0	0.0	0.0	0.0	4.0	0.0	0.0	0.0	12.2	0.4	0.0	0.0
94	0.0	17.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cult. Slds.	0.0	3.8	2.5	0.0	2.4	0.0	0.4	0.0	1.7	4.8	2.1	0.0	

Trials With Taka-Diastase

Having this indication that the addition of diastase in the form of sputum to the medium stimulated germination, Taka-diastase was added to medium No. 2 after the agar was melted and ready for use, i.e., immediately before each test. This medium is designated as No. M2D10. The results of the first trial are shown in Table IX:

Table IX

Showing the effect of Taka-diastase (added to medium No. 2 at the rate of one drop to 10 ml. of agar) on the percentages of germination of pollen of the variety H 456 at the relative humidities and pH values indicated and at 23.0° C.

pH

	5.2		5.8		6.1		
R. H.	M 2	M 2 D10	M 2	M 2 D10	M 2	M 2 D10	Control
100%	7.5	0.0	0.0	0.0	0.0	0.0	0.0
98%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
96%	0.0	0.0	8.8	9.0	6.7	0.0	0.0
94%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cult. Slds.	2.7	0.0	0.0	0.0	0.0	0.0	

It is apparent from the data contained in Table IX that fewer germinations occurred in the medium to which the Taka-diastase was added. This test was repeated under the same conditions of temperature, humidity and pH value of medium, using pollen from the variety D 1135 with similar results. The question arises from the results of these two tests as to whether this inhibitive effect was the result of the direct action of the diastase on the pollen grains themselves, or was the result of the presence of dextrose into which some of the sucrose was changed by the action of the diastase, or was simply the result of an improper amount of diastase. By adding a smaller amount of Taka-diastase (one drop to 20 ml. of the agar), there were considerably more germinations in this medium than in the preceding test. When one drop of 10 per cent Taka-diastase was added to the same amount of agar, a still higher per cent of germination resulted but in each case so far these per cents were less than those in the medium without the diastase.

To separate test tubes, each containing 10 ml. of medium No. 2 was added 1 drop of 2, 4, 6 and 8 per cent Taka-diastase. These dilutions of Taka-diastase were made with sterile distilled water. Pollen of the variety H 456 was used. The test was run in the same manner as the preceding ones with the results shown in Table X:

TABLE X

Showing the effect on the percentages of germination of H 456 pollen of the additions of Taka-diastase to medium No. 2 at the rate of 1 drop of 2, 4, 6 and 8 per cent as against no diastase (M2); the pH value of the medium being 5.2; the temperature, 23.5° C.; and the relative humidity being 100 per cent and 98 per cent.

R. H.	M 2	2% T-d (1)	4% T-d	6% T-d	8% T-d	Controls (2)
100%	0.9	1.9	2.8	4.8	5.0	0.3
98%	0.8	5.1	6.7	4.5	4.3	0.0
Cult. Slds.	1.3	0.0	3.5	1.7	1.2	

(1) T-d=Taka-diastase.
(2) Average of 3 vials for each humidity.

There is a definite indication from the data of Table X that stimulation of germination resulted from additions of Taka-diastase. In every case where the diastase was added, there was an increased per cent of germination over that occurring in the medium No. 2. Furthermore, the percentages of germination increased as the per cent of diastase added increased. The failures of some and the inhibitive effect of others of the preceding tests with Taka-diastase may, therefore, be explained on the basis of unsuitable amounts having been added.

TRIALS WITH RAW SUGAR

It has been found by Brink (2) that higher percentages of pollen germination occur on culture media made with commercial granulated sugar than on those made with C. P. sucrose (2, p. 292).* The explanation of this is suggested by the fact that certain substances, perhaps organic, present in the commercial product are absent in the chemically pure sucrose. It was decided, therefore, to carry this idea one step further in the direction of the impure sugar. Raw sugar which had been washed once in the milling process was, therefore, used in making up a medium. This medium was made up in the same way in every respect as medium No. 2 and is designated as M2R. A test was made with H 456 pollen using this medium and medium No. 2, the results of which are shown in Table XI.

TABLE XI

Showing the percentages of germination of H 456 pollen obtained in a medium made with raw sugar (M2R) as compared with medium No. 2 at 20° C. and at the relative humidities and pH values indicated.

* Brink's paper available to the author at this point.

pH

	5.2		5.8		6.1		
R. H.	M 2	M 2 R	M 2	M 2 R	M 2	M 2 R	Control (1)
100%	3.5	0.0	5.5	0.0	1.7	0.0	0.0
98%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
96%	0.0	0.0	0.0	0.0	1.0	0.0	0.0
Cult. Slds.	0.0	0.0	0.0	0.0	0.0	0.0	

(1) Average of four vials for each humidity.

It is evident from these figures that raw sugar did not increase pollen germination but definitely inhibited it. Several tests were run at higher temperatures with this medium but with similar results.

The pH Values of the Media Adjusted With Various Acids

In the next test the pH values of agar were adjusted by the use of three different acids: sulphuric (M2S), phosphoric (M2P), and malic (M2M). Agar No. 2 was used. The pH values were adjusted to 5.2, 5.8 and 6.1. Pollen of the variety H 456 was used and the temperature was 24.0° C. The results of this test are shown in Table XII.

Table XII

Showing the effects on the percentages of germination of H 456 pollen of agars having their pH values adjusted by sulphuric, phosphoric, and malic acids to the indicated reactions, at the indicated relative humidities, and at 24.0° C.

pH

	5.2			5.8			6.1			
R. H.	M2S	M2P	M2M	M2S	M2P	M2M	M2S	M2P	M2M	Control (2)
100%	9.5	0.0	0.0	6.2	4.3	10.0	0.0	0.0	2.5	5.8
98	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
96	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cult. Slds.	16.6	0.0	0.0	0.0	0.0	0.0	16.6	0.0	0.0	

(2) Average of three vials for each humidity.

It is seen from the data of Table XII that when the pH value of the medium was adjusted to 5.2 with sulphuric acid the percentage of germination was 9.5 at 100 per cent R.H. and 16.6 in the culture slides. When the same adjustment was made with phosphoric and malic acids no germinations resulted. When the pH value was adjusted to 5.8 with malic acid, higher percentages of germination were obtained than when this adjustment was made with sulphuric acid or phosphoric acid. When the reaction of the medium was adjusted to 6.1 with sulphuric acid and malic acid, the percentages of germination were respectively 16.6 in the culture slides, and 2.5 at 100 per cent relative humidity. These tests were repeated several times with similar results. When the reaction of the medium was adjusted

to 6.1 with these three acids the results were sometimes in favor of one and sometimes in favor of another.

Comparison of Germinations on Successive Days After Tassels Were Cut

In the next three tests, a comparison was made of the percentages of germination of pollen from tassels kept with their cut ends in sulphurous acid for one, two and three days. Six tassels of the variety H 456 were used for this purpose. The pollen used in these tests was a composite sample of all six tassels. The pH values of agar No. 2 were adjusted to 5.2, 5.8, and 6.1 with sulphuric, phosphoric and malic acids. Both the culture slide and the vial methods were used. For the vials the percentages recorded in Table XIII represent the averages of two counts. The relative humidity in the vials was maintained at 100 per cent. The temperature was 24.0° C. The results of these three tests are shown in Table XIII.

Table XIII

Showing the relative percentages of germination of H 456 pollen on the first three days after the tassels were cut, composite samples of pollen from six tassels being used, at the pH values indicated, at the relative humidity of 100 per cent and in culture slides, and at 24.0° C. The pH values were adjusted by sulphuric acid (M2S), phosphoric acid (M2P), and malic acid (M2M).

	pH								
	5.2 Day			5.8 Day			6.1 Day		
R. H.	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
				M 2 S					
100%	3.0	16.6	0.0	6.4	5.3	0.0	6.6	2.2	0.0
Cult. Slds.	5.4	8.2	0.0	9.6	8.8	0.0	15.0	6.1	0.0
				M 2 P					
100%	0.6	2.2	0.0	2.3	6.9	0.0	0.0	0.3	0.0
Cult. Slds.	2.2	1.0	0.0	8.4	10.9	0.0	1.6	0.0	0.0
				M 2 M					
100%	1.3	2.4	0.0	2.6	7.5	0.0	1.2	4.2	0.0
Cult. Slds.	5.7	0.0	0.0	6.9	11.6	0.0	6.4	3.6	0.0

From the data of Table XIII it is seen that on the first day the highest per cent of germination in the vials was 6.6 and occurred in medium M2S adjusted to a pH value of 6.1; in the culture slides it was 15.0 per cent and likewise occurred in M2S at a pH value of 6.1. On the second day the highest per cent of germination in the vials was 16.6 occurring in M2S at a pH value of 5.2; in the culture slides, it was 11.6 occurring in M2M at a pH value of 5.8. On the third day, no germination occurred at all.

These results agree with those previously reported (4), namely, that the percentage of germination of pollen from cut tassels decreases rapidly on successive days after the tassels are cut.

Here again at a pH value of 5.2 the highest per cent of germination occurred in the medium adjusted with sulphuric acid. At a pH value of 5.8 it occurred

in the medium adjusted with malic acid and, at a pH value of 6.1, the highest per cent of germination occurred, in the medium adjusted with sulphuric acid. Whenever the pH values were adjusted with phosphoric acid the percentages of germination have been consistently lower than when the adjustments were made with either sulphuric acid or malic acid.

Temperature

The data shown in Tables VI, VIII, V, IV, IX, and XI seem to indicate that temperature has a decided influence on the percentages of germination of sugar cane pollen. These tables show the percentages of pollen of the variety H 456 germinating on medium No. 2 at temperatures ranging from 27.0° C. to 20.0° C. The maximum percentage of germination of each of these tables is shown in Table XIV.

Table XIV

Showing the maximum percentage of germination (Tables VI, VIII, V, IV, IX, and XI) of H 456 pollen obtained on medium No. 2 at temperatures ranging from 27.0° C. to 20.0° C.

Per Cent Germination	Temperature of Oven ° C.	Air Temperature at Time of Pollen Shed ° C.	No. of ° C. Oven Was Above or Below Air Temperature	Table
0.8	27.0	21.1	+5.9	VI
3.1	26.0	23.6	+2.4	VIII
3.4	25.5	23.8	+1.7	V
5.5	24.5	23.6	+0.9	IV
8.8	23.0	22.2	+0.8	IX
5.5	20.0	22.7	—2.7	XI

It is seen from the above table that the maximum percentage of germination was 8.8 per cent and occurred at 23.0° C. Previous tests without agar indicated that the optimum temperature was somewhere near 22.0° C. (4). It is seen further from Table XIV that there is a correlation between the percentage of germination and the temperature at which the germination occurred. However, when the difference between the temperature of the air at which the pollen was shed and the temperature at which the germination of the pollen took place is taken into consideration, it is seen that there exists another correlation which is equally tenable.

While the minimum germination (0.8 per cent) occurred at 27.0° C., there was as much as 5.9° C. difference between the temperature of the air when the pollen was discharged from the anthers and the temperature at which germination of this pollen took place in the oven. The maximum germination (8.8 per cent) occurred at 23.0° C. The difference between this temperature and that of the air when the pollen was shed was only 0.8° C. In other words, the maximum percentage of germination occurred at the temperature differing the least from that of the temperature of the air when the pollen was shed and the minimum percentage occurred when these two temperatures differed the most.

Discussion

As to why the requirements for the germination of sugar cane pollen are so exacting in regard to an artificial medium, it is not easy to explain. According to Brink (2): "A source of no little annoyance and perplexity to those who have attempted to cultivate pollen artificially has been the bursting of pollen and pollen tubes. No satisfactory explanation of this phenomenon has as yet been brought forward." He cannot agree with Van Tieghem, Molisch, Lidforss, and others that the bursting of pollen grains and pollen tubes "is not related to the osmotic force of the surrounding medium," and says that their conclusion "that it is not an unbalanced osmotic condition . . . has been based on the assumption that the protoplasmic surface of the pollen grain acts as a semi-permeable membrane to sugar solutions. There is no good evidence to show that this, in general, is the case." He feels rather that the evidence "favors the view that it is largely an osmotic phenomenon" and then brings forward considerable evidence to show that "pollen tubes of many species will grow readily on sugar solutions varying widely in concentration" and arrives at the conclusion that "the cell membranes of the pollen grain and its tube are, or become, permeable to cane sugar, and that the final result as far as osmotic pressure is concerned is the same as though the surrounding medium were water. Perhaps osmotically active substances are produced within the cell, bringing it into equilibrium with the particular concentrations of sugar without. If this were so, we should expect a well-defined optimum concentration of sugar for early growth, above which the pollen would plasmolyze and below which, if the wall could not sustain the pressure, the tubes would burst."

From the fact that in the media used in these tests bursting occurred in the low concentrations and plasmolysis in the high ones it would seem that plasmolysis and bursting were due to osmotic pressure and that the cell wall of the sugar cane pollen at least is semi-permeable. Anthony and Harlan found that barley pollen burst in low concentrations of sugar solutions and plasmolyzed in high ones (1).

In previous tests also (4) there were indications that the pollen tube is semi-permeable for evidence of osmotic pressure is seen in the swelling of the pollen tubes (Fig. 3), which can hardly be explained on the basis of imbibition alone. It is seen further that the walls of these tubes become thinner at their distal ends as is evidenced by the position of the swellings and final ruptures.

Medium No. 1 was of semi-liquid consistency (*sol*). Medium No. 2 was slightly more viscous and of a consistency that might be considered either a *sol* or a *jel*. Medium No. 3 was more viscous than medium No. 2, or a semi-solid (*jel*), and continuing on through media Nos. 4, 5, 6 and 7 there was a gradation of increasing viscosities.

This gradation of viscosities in the series of media was due principally to increased percentages of agar although it was influenced also by increased percentages of sugar. Being emulsoids it is seen that, as the disperse phase is increased in this series, the amount of dispersion medium in each was relatively decreased. This means that, beginning with medium No. 1 and continuing on through the series, in the last one of this series the structure of the original emulsoid was partially, if not completely, reversed and that in medium No. 1, for example, the concentration of the sugar solution available to the pollen grain and tube was higher

than the percentage indicated in Table I and that between each medium and the succeeding one there was a greater increase in sugar concentration than is indicated by the figures of Table I.

When using sulphuric acid solutions in sealed chambers to control relative humidity the concentrations of the media used would either remain the same or change according to whether the hanging drops took up or gave off moisture as influenced by the relative humidity of the air surrounding them. Thus the germination of pollen grains, if lying on the surface of the medium, would not only be influenced directly by the relative humidity of the air with which they were in contact, but also it would be influenced indirectly by any resultant change in the concentration of agar and sugar of the medium used; if lying beneath the surface, it would be influenced only indirectly.

The fact that in medium No. 2 the maximum per cent of germination occurred and that the tubes of these pollen grains at the optimum temperature, humidity, etc., were characterized by exceptional length (Fig. 2, D) indicates that this medium resulted in the optimum rate of osmosis or imbibition. As the pollen tube developed and advanced through the medium, the sugar solution (dispersion medium) was absorbed by osmosis or imbibition. If, as in medium No. 2, let us suppose, this absorption *rate* was balanced with the *rate* of increasing capacity of the *growing* tube, and if the actual amount of food so absorbed in this particular concentration of sugar solution met the requirements for *growth* of the developing pollen tube, the maximum per cent of germination and the maximum tube length would result. This rate of absorption would not only depend upon the osmotic pressure due to the concentration of sugar in the dispersion medium but also the structure of the interlacing phases of the emulsoid would control the amount of the disperse phase with which the growing tube would come in contact. If, in the case of medium No. 1, we had a *sol* with concentrated droplets of the disperse phase scattered through the dispersion medium so that the absorption rate was greater than the rate of increasing capacity of the growing tube, bursting would result even before a tube was actually developed by the pollen grain. If, as in media Nos. 4 to 7, the process of osmosis or imbibition was reversed, plasmolysis resulted.

Summary

From the results of the preceding experiments together with those previously reported, from those reported by Dutt and Ayyar, and others, it is evident that the germination of sugar cane pollen is dependent upon a nice balance of retroreactive physical and chemical factors.

An attempt is made in these experiments to standardize a method of germinating sugar cane pollen by controlling the concentration of sugar and agar in hanging drops of culture media, the pH value of the media, the relative humidity and the temperature of the air surrounding the hanging drops, and by the addition of enzymes to the culture media.

Quantitative data are presented to show that, when a series of culture media having a gradient of viscosities due to increasing concentrations of sugar and agar were used, a particular medium consistently gave the maximum percentage of germination. These percentages consistently exceeded those of the controls.

An optimum relative humidity of 96 per cent and an optimum temperature of approximately 23.0° C. were demonstrated.

While there was a correlation between temperature and percentage of germination there also existed a correlation between percentage of germination and the difference between the temperature of the air when the pollen was shed and the temperature at which germination occurred.

When the pH values of the media were adjusted with sulphuric, phosphoric, and malic acids, better results were obtained with sulphuric acid. The optimum pH value was approximately 5.2.

Definite stimulation of germination was demonstrated by the addition of definite amounts of Taka-diastase to the culture medium.

The percentage of germination of pollen from cut tassels decreased from 16.6 per cent on the second day to 0.0 per cent on the third day after cutting.

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Sugar Prices

96° Centrifugals for the Period March 17 to June 13, 1930

Date	Per Pound	Per Ton	Remarks
Mar. 17, 1930	3.625¢	$72.50	Philippines, 3.64, 3.61.
" 18	3.61	72.20	Philippines, Porto Ricos, Cubas.
" 19	3.595	71.90	Philippines, 3.58; Cubas, 3.58, 3.61.
" 22	3.52	70.40	Porto Ricos.
" 26	3.58	71.60	Philippines.
" 27	3.61	72.20	Porto Ricos, Philippines.
" 28	3.655	73.10	Porto Ricos, 3.67, 3.64; Cubas, 3.64.
" 31	3.64	72.80	Porto Ricos.
April 1	3.595	71.90	Porto Ricos, 3.58, 3.61; Cubas, 3.61.
" 2	3.625	72.50	Cubas, 3.61; Porto Ricos, 3.61, 3.64; Philippines, 3.61.
" 4	3.623	72.47	Porto Ricos, 3.61; Philippines, 3.61; Cubas, 3.64, 3.62.
" 5	3.52	70.40	Cubas, Porto Ricos.
" 7	3.49	69.80	Porto Ricos.
" 8	3.46	69.20	Cubas.
" 9	3.433	68.66	Cubas, 3.39, 3.45, 3.46.
" 15	3.42	68.40	Cubas.
" 24	3.405	68.10	Cubas, 3.39; Porto Ricos, 3.42.
" 26	3.39	67.80	Cubas.
" 29	3.33	66.60	Cubas.
May 1	3.30	66.00	Cubas.
" 6	3.27	65.40	Cubas, Philippines.
" 9	3.24	64.80	Cubas.
" 12	3.225	64.50	Cubas, 3.24, 3.21.
" 13	3.18	63.60	Philippines, 3.19, 3.17.
" 15	3.182	63.65	Philippines, 3.155; Cubas, 3.21.
" 16	3.24	64.80	Philippines.
" 19	3.21	64.20	Cubas, Philippines.
" 21	3.19	63.80	Cubas, 3.21; Porto Ricos, 3.17.
" 22	3.17	63.40	Porto Ricos.
" 23	3.14	62.80	Philippines.
" 24	3.425	68.50	Cubas, 3.39; Porto Ricos, 3.46.
" 27	3.132	62.65	Cubas, 3.14; Porto Ricos, 3.125.
" 29	3.14	62.80	Philippines.
June 4	3.225	64.50	Cubas, 3.21; Philippines, 3.21; Porto Ricos, 3.24.
" 5	3.24	64.80	Philippines, Porto Ricos, Cubas.
" 6	3.26	65.20	Porto Ricos, 3.27; Cubas, 3.27, 3.25; Philippines, 3.27.
" 10	3.255	65.10	Porto Ricos, 3.27; Cubas, 3.24.
" 11	3.27	65.40	Porto Ricos.
" 13	3.315	66.30	Cubas, 3.30, 3.33; Philippines, 3.33.